



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

#13

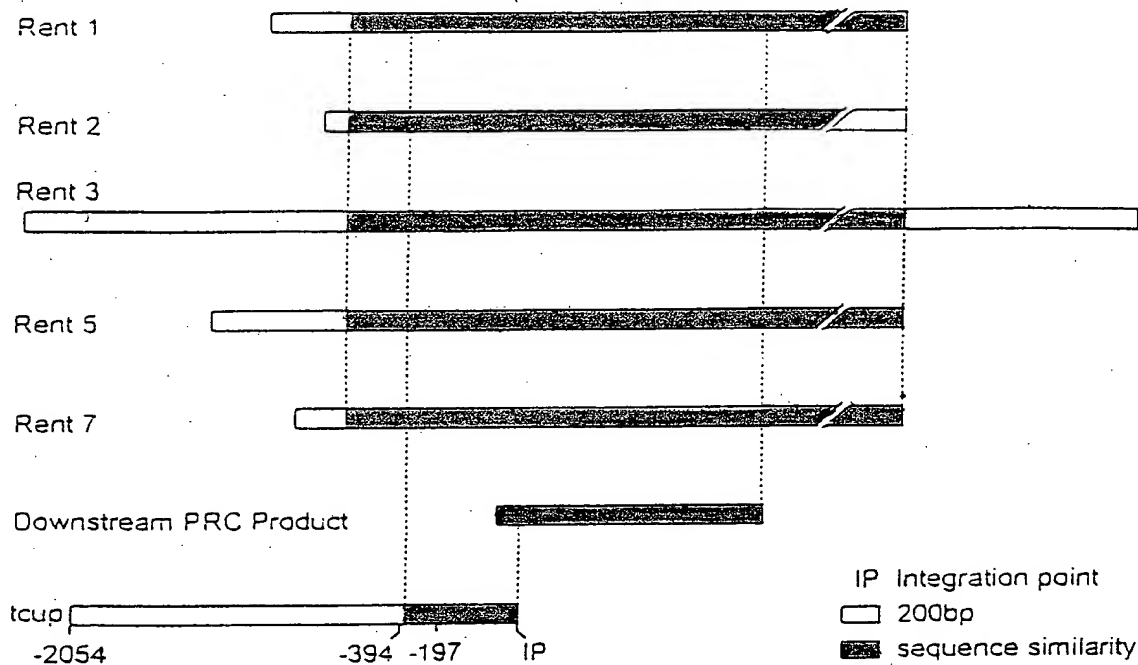


FIGURE 1A



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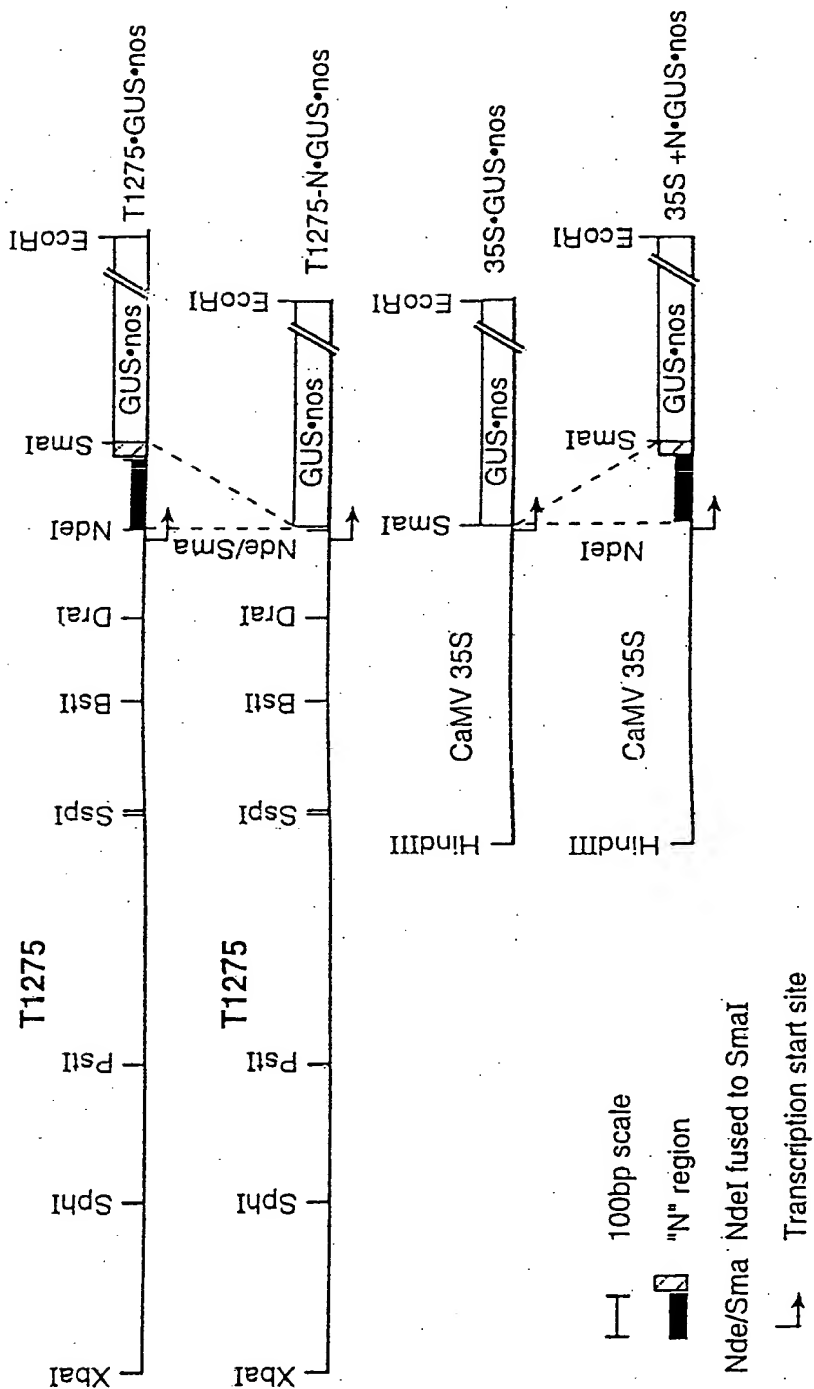


Figure 1B



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	10	20	30	40	50
tCUP	1	-----	-----	-----	-----
Rent1.	1	-----A-	-----	ATTGTAAGCG	GGATAACAAT
RENT2.	1	-----AT	GTTGTGTGGA	ATTGTGAGCG	GGATAACAAT
RENT3.	1	TT-----	-----	-----	-----
RENT5.	1	-----	-----GGA	ATTGTGAGCG	G-ATAACAAT
RENT7.	1	TTTATGCTTC	CGGCTCGTAT	GTTGTGTGGA	ATTGTGAGCG G-ATAACAAT

	60	70	80	90	100
tCUP	51	-----	-----	-----	-----
100 Rent1.	51	TTCACACAGG	AAACAGCTAT	GACCATGATT	ACGCCAAGCT TTTAATACGA
100 RENT2.	51	TTCACACAGG	AAACAGCTAT	GACCATGATT	ACGCCAAGCT CT-AATACGA
100 RENT3.	51	-----	-----	-----	-----
100 RENT5.	51	TTCACACAGG	AAACAGCTAT	GACCATGATT	ACGCCAAGCT CT-AATANGA
100 RENT7.	51	TTCACACAGG	AAACAGCTAT	GACCATGATT	ACGCCAAGCT CT-AATACGA
100					

	110	120	130	140	150
tCUP	101	-----TTA	TAATTACAAA	ATTGATTCTA	GTATCTTTAA
150 RENT1.	101	CTCACTATAG	GGAAAGCTTA	TAATTACAAA	ATTGATTCTA GTATTTTTTAA
150 RENT2.	101	CTCACTATAG	GGAAAGCTTA	TAATTACAAA	ATTGATTCTA GTATTTTTTAA
150 RENT3.	101	-----	-----	---GATTCTA	GTTTTTTTTAA
150 RENT5.	101	CTCACTATAG	GGAAAGCTTA	TAATTACAAA	TTTGATTCTA GTATTTTTTAA
150 RENT7.	101	CTCACTATAG	GGAAAGCTTA	TAATTACAAA	ATTGATTATA GTACTTTTAA
150					

Figure 1C1



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tCUP	150	170	180	190	200	
200	151	TTTAATGCTT	ATACATTATT	AATTAATTTA	GTACTTTCAA	TTGTTTTCA
RENT1.	151	TTTAATATTT	TTACATTATT	AATTAATTTA	GAAGTTTTAA	TTTTTTTTCA
200						
RENT2.	151	TTTAATATTT	ATACATTATT	AATTAACCTA	GTACTTTCAA	TTGTTTTCA
200						
RENT3.	151	TTTAATATTT	ATACATTATT	AATTAATTTA	GTTCTTTCAA	TTGTTTTCA
200						
RENT5.	151	TTTAATATTT	ATACATTATT	AATTAATTTA	GTACTTTCAA	TTGTTTTCA
200						
RENT7.	151	TTTAATATTT	ATACATTATT	AATTAATTTA	GCACTTTCAA	TTTATTTTCA
200						
tCUP	210	220	230	240	250	
250	201	GAAATTATTT	TACTATTTTT	TATAAAATAA	AAGGGAGAAA	ATGGCTATTT
RENT1.	201	GAAATCATTT	TACTATTTTT	-ATAAAAACA	AAAGGGAAAA	GTGGTTATTT
250						
RENT2.	201	AAAATTATTT	TACTATTTTT	TGTAAAATAA	AAGGGAGAAA	ATGGCTATTT
250						
RENT3.	201	GAAATTATTT	TACTATTTTT	TATAAAATAA	AAGGGAGAAA	ATGGCTATTT
250						
RENT5.	201	GAAATCATTT	TACTATGGTT	TATAAAATAA	AAGGGAGAAA	ATGGCTATTT
250						
RENT7.	201	GAAACCATTT	TACTATTTTT	TATAAAATAA	AAGGGACAAA	ATGGCTATTT
250						
tCUP	260	270	280	290	300	
300	251	AAATACTAGC	-CTATTTTAT	TTCAATTTTA	GCCTAAAATC	AG-CCCCAAT
RENT1.	251	AAATACTAGC	CCTATTTTCAT	TTCAATTATA	GCCTAAAATC	AGCCCC-AAT
300						
RENT2.	251	AAATACTAGC	CCTATTTTAT	TTCAATTTTA	GCCTAAAATC	AGCCCCCAAT
300						
RENT3.	251	AAATACCAGC	CCTATTTTAT	TTCAATTTTA	ACCTAAAATC	AGCCCC-AGT
300						
RENT5.	251	AAATACTAGC	CCTATTTTAT	TTCAATTTTA	GCCTAAAATC	AGCCCC-AAT
300						
RENT7.	251	AAATACCAAC	ACTATTTTAT	TTCAATTTTA	GCCTAAAATC	AAACCC-AAT
300						

Figure 1C2



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	310	320	330	340	350
tCUP	301	TAGCCCCAAT	TTCAAATTCA	AATGGTCCAG	CCCAATTCCT AAA-TAACCC
350					
RENT1.	301	TAACCCCCAAT	TCCAAATTCA	AACGGGCCAG	CCCAATTCCT AAAATGACCC
350					
RENT2.	301	TAACCCCCAAT	TTCAAATTCA	AATGGGACAG	CCCAATTCCT AAAATAACCC
350					
RENT3.	301	TAGCCCC--	-----A	AACGGCCCAT	CCCAATTCCT AAAATAACTC
350					
RENT5.	301	TAACCCCTAT	TTCAAATTCA	AACGGGCTAG	CCCAGTTCCT AAAATAACCC
350					
RENT7.	301	TAACCCC--	-----A	AACGGGCCAG	CCCAATTCCT AAAACAACCC
350					

	360	370	380	390	400
tCUP	351	ACCCCTAACC	C-----	-----GCCCGG	TTTCCCCTTT TGATCCAGGC
400					
RENT1.	351	GCTCCTAACC	CGCTTTTCCA	ACCCGCCCGG	TTTCCCCTTT TGATCCAGGC
400					
RENT2.	351	GCCCCTAACC	CTCTTATCCA	ACCCACCCGA	TTTCCCCTTT TGATCCAGGT
400					
RENT3.	351	GCCCCTAACC	CGCTTATCCA	ACCCGCCCGG	TTCCC-CTTT TGATCCAGGC
400					
RENT5.	351	TCCCCTAACC	CGCTTATCCA	ACCCGCCCTG	TTTCCCCTTT TGATCCAGGC
400					
RENT7.	351	GCCCCTAACC	CGCTTATCCA	ACCCGCCCGA	TTTCTCTTT TGATCCAGGC
400					

	410	420	430	440	450
tCUP	401	CGTTGATCAT	TTTGATCAAC	GCCCAGAATT	TCCCCTTTTC CTTTTTTAAT
450					
RENT1.	401	TGTTGATCAT	TTTGATCAAC	GGCCAGAATT	TCCCCTTTTC --TTTTTAAT
450					
RENT2.	401	TGTTGATCAT	TTTGATCAAC	GACCAGAATT	TCCCCTTTTC TGTTTTTAAT
450					
RENT3.	401	CGTTGATCAT	TTTGATCAAC	GACCAGAATT	TCCCCTTTTC -TTTTTTAAT
450					
RENT5.	401	CGTTGATCAT	TTTGATCAAC	GACCAAAATT	TCCCCTTT-C CTTTTTTAAT
450					
RENT7.	401	CGTTGATCAT	TTTGATCAAC	GGCCAGAATT	TCCCCTTTTC -TTTTTTCAT
450					

Figure 1C3



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	460	470	480	490	500
tCUP	451	TCCCAAACAC	C-CCTAACTC	TATCCCATT	CTCACCAACC GCCACATATG
500					
RENT1.	451	TCCCAAACAC	CCCCCAACCT	TATCCCGTTT	CTCACCAACC GCCAGATCT-
500					
RENT2.	451	TCCCAAACAC	CCCCCAACCC	TATCCCATT	CTCACCAACC GCCAGATCT-
500					
RENT3.	451	TCCCAAACAC	CGCC-AAACC	TATCCCATT	CTCACCAACC GCCAGATCTA
500					
RENT5.	451	TCCCAAACAC	CCCC-AACCC	TATCCCATT	CTCACCAACC GCCAGATCT-
500					
RENT7.	451	TCCCAAACAC	CCCC-AAACC	TATCCCATT	CTCACCAACC GCCAGATCTA
500					
	510	520	530	540	550
tCUP	501	AATCCTCTTA	TCTCTCAAAC	TCTCTCGAAC	CTTCCCCTAA CCCTAGCAGC
550					
RENT1.	501	-ATCCTCTTA	TCTCTCAAAC	TCTCTCGAAC	CTTCCCCTAA CCCTAGCAGC
550					
RENT2.	501	-ATCCTCTTA	TCTCTCAAAC	TCTCTCGAAC	CTTCCCCTAA CCCTAGCAGC
550					
RENT3.	501	T--CCTCTTA	TCTCTCAAAC	TCTCTCGAAC	CTTCCCCTAA CCCTAGCAGC
550					
RENT5.	501	-ATCCTCTTA	TCTCTCAAAC	TCTCTCGAAC	CTTCCCCTAA CCCTAGCAGC
550					
RENT7.	501	T--CCTCTTA	TCTCTCAAAC	TCTCTCGAAC	CTTCCCCTAA CCCTAGCAGC
550					
	560	570	580	590	600
tCUP	551	CTCTCATCAT	CCTCACCTCA	AAACCCACCG	GAATACATGG CTTCTCAAGC
600					
RENT1.	551	CTCTCATCAT	CCTCACCTCA	AAACCCACCG	GCCACCATGG CCTCTAGAG-
600					
RENT2.	551	CTCTCATCAT	CCTCACCTCA	AAACCCACCG	GCCACCATGG CCTCTAGAG-
600					
RENT3.	551	CTCTCATCAT	CCTCACCTCA	AAACCCACCG	GCCACCATGG CCTCTAGAGG
600					
RENT5.	551	CTCTCATCAT	CCTCACCTCA	AAACCCACCG	GCCACCATGG CCTCTAGAG-
600					
RENT7.	551	CTCTCATCAT	CCTCACCTCA	AAACCCACCG	GCCACCATGG CCTCTAGAGG
600					

Figure 1C4



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	610	620	630	640	650
tCUP	601	CGTGGAACC	TTATACTCAC	CTCCCTTTGC	TCTTACAGTA CTC-GGCCGT
650					
RENT1.	601	---GATCCCC	GGGTGGTCAG	TCCCTTATG-	--TTAC---- -----GT
650					
RENT2.	601	----GA----	-----	-TCCCCG---	-----GGTG GTC-AGTCCC
650					
RENT3.	601	ATC----CCC	GGGTGGTCAG	TCCCTTATGT	NA-----CG NCCTAAATGN
650					
RENT5.	601	---GATCCCC	GGGTGGTCAG	TCCCTTATG-	--TTACG--- -----
650					
RENT7.	601	ATC----CCC	GGGTGGTCAG	TCCCTTATGT	TA-----CG TCCTN-----
650					

	660	670	680	690	700
tCUP	651	CGACCGCGGT	ACCCGGG...
700					
RENT1.	651	C-----	--CTNAA...
700					
RENT2.	651	TTAT-GTG--	---CGTC...
700					
RENT3.	651	CCGNCCTGNN	NNNNN-C...
700					
RENT5.	651	-----	TCC-----
700					
RENT7.	651	-----	-----
700					

Figure 1C5



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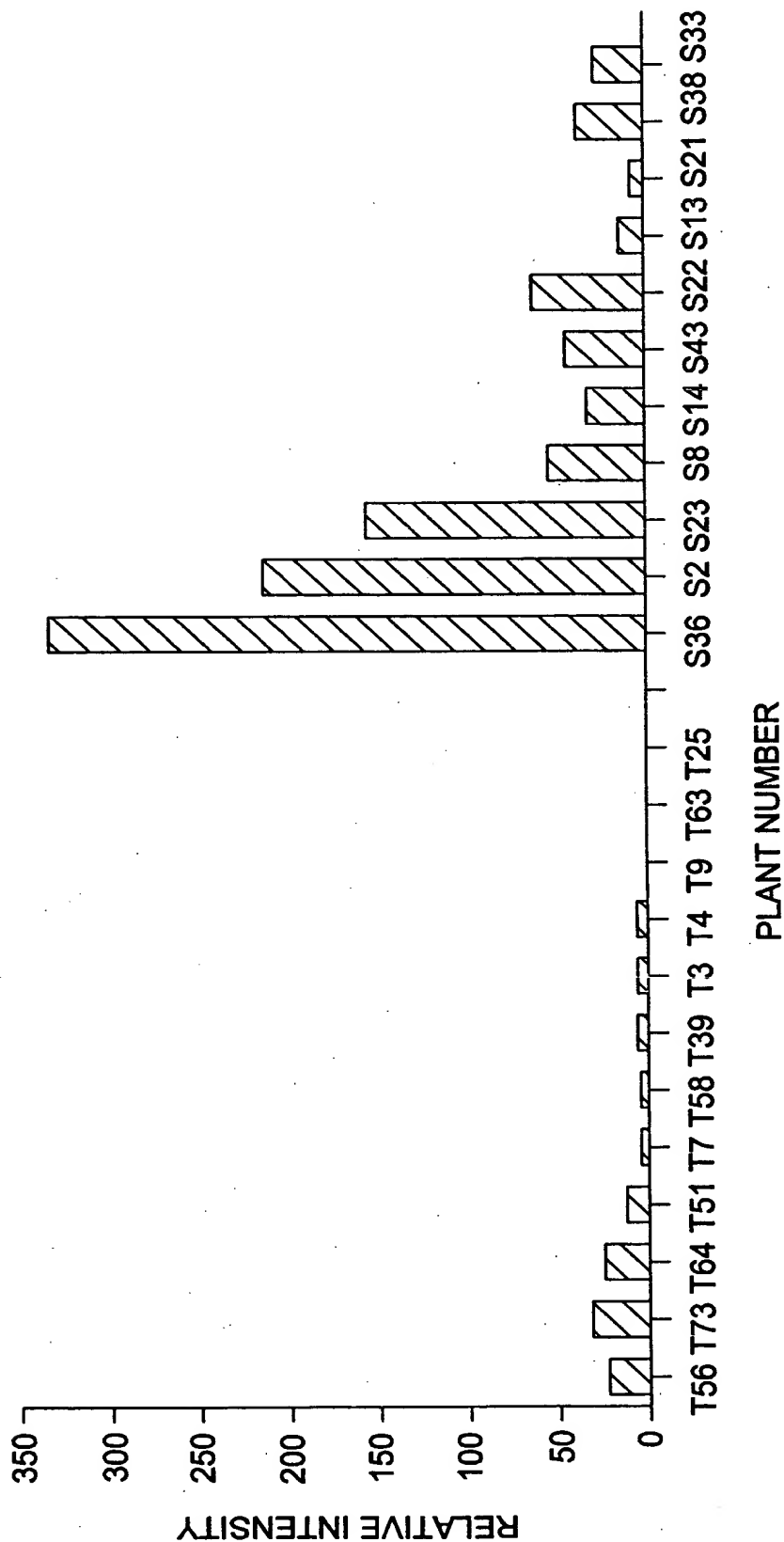


Figure 2A



Title: TRANSLATIONAL REGULATORY ELEMENTS
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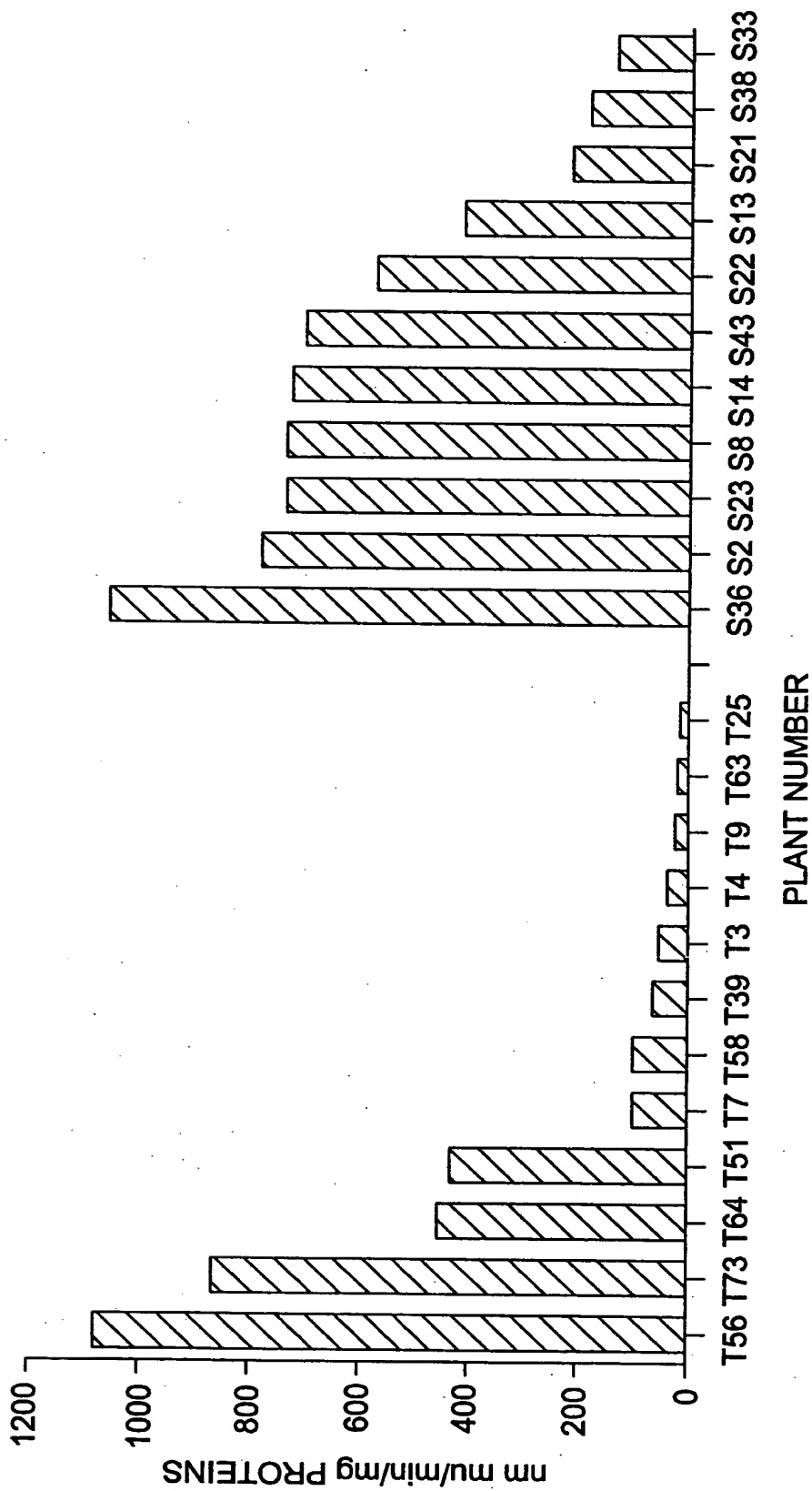


Figure 2B



Title: TRANSLATIONAL REGULATORY ELEMENTS

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Application No.: 09/747,007

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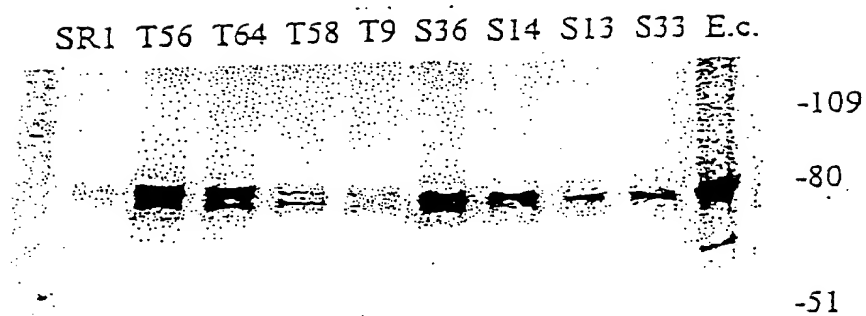


FIGURE 2C



Title: TRANSLATIONAL REGULATORY ELEMENTS

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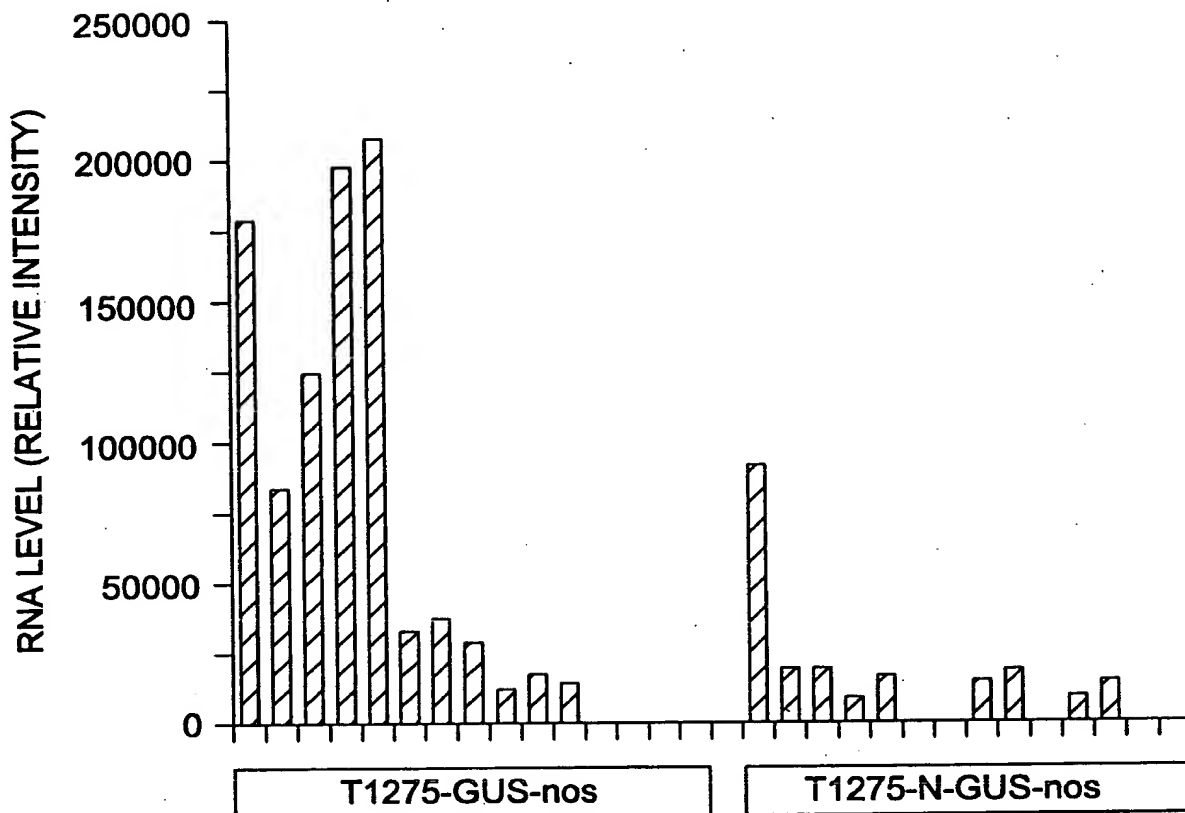


Figure 3A

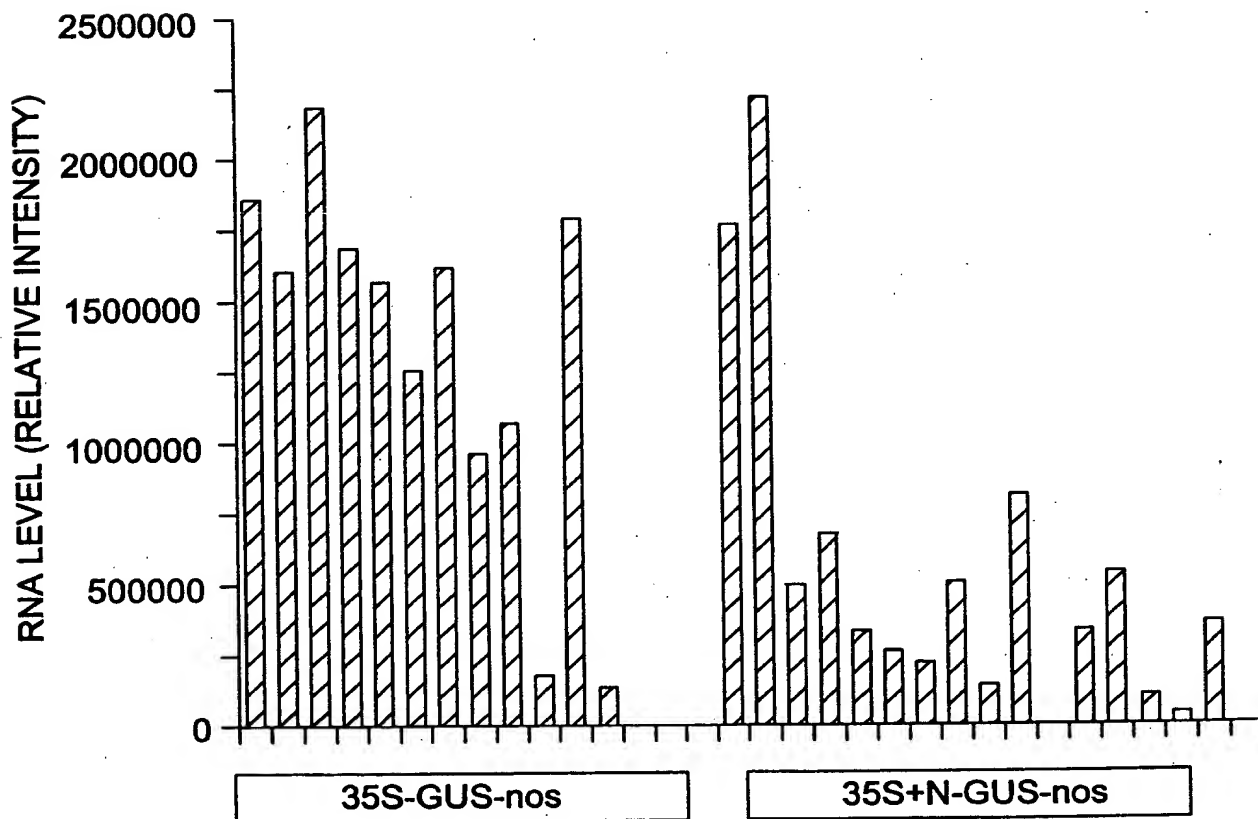


Figure 3B



Title: TRANSLATIONAL REGULATORY ELEMENTS

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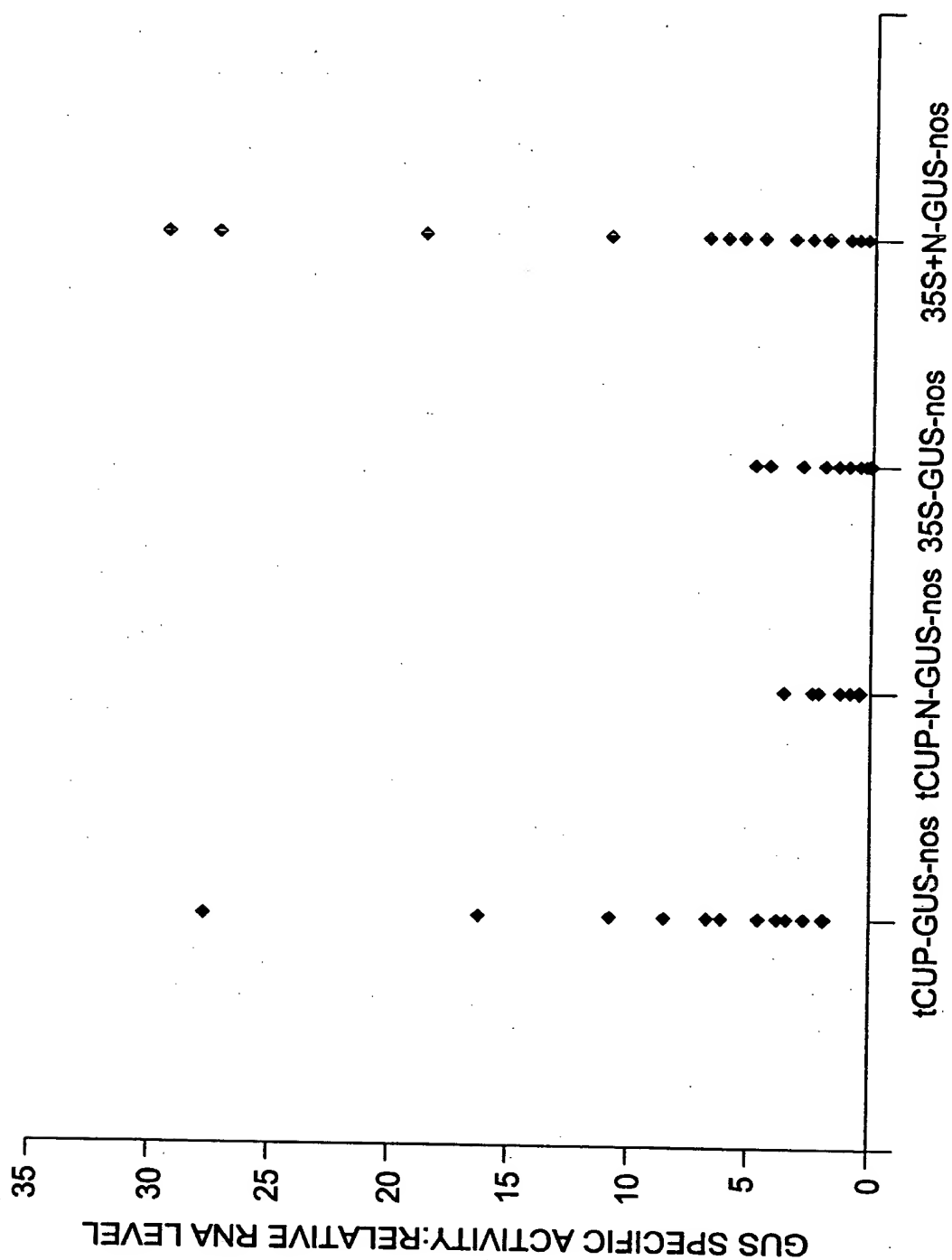


Figure 3C



Title: TRANSLATIONAL REGULATORY ELEMENTS

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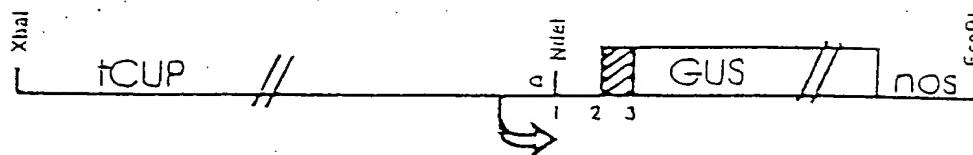
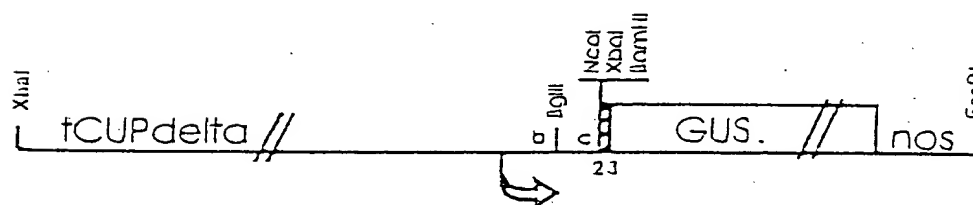


FIGURE 4A



a NdeI position +30 $\overline{\text{ACA TAT GAA}}$
 b BglII position +29 $\overline{\text{ACA GAT CT}}$
 c NcoI position 0 $\overline{\text{CC ACC ATG GCC TCT AGA GGA TCC}}$ $\overline{\text{CCG GGT GGT CAG TCC CTT ATG}}$
 tCUP initiation site $\overline{\text{GAA TAC ATG G}}$ /... tCUP leader .../ $\overline{\text{CCG GGT GGT CAG TCC CTT ATG}}$
 Kazak consensus $\overline{\text{CC ACC ATG G}}$

FIGURE 4B



Title: TRANSLATIONAL REGULATORY ELEMENTS

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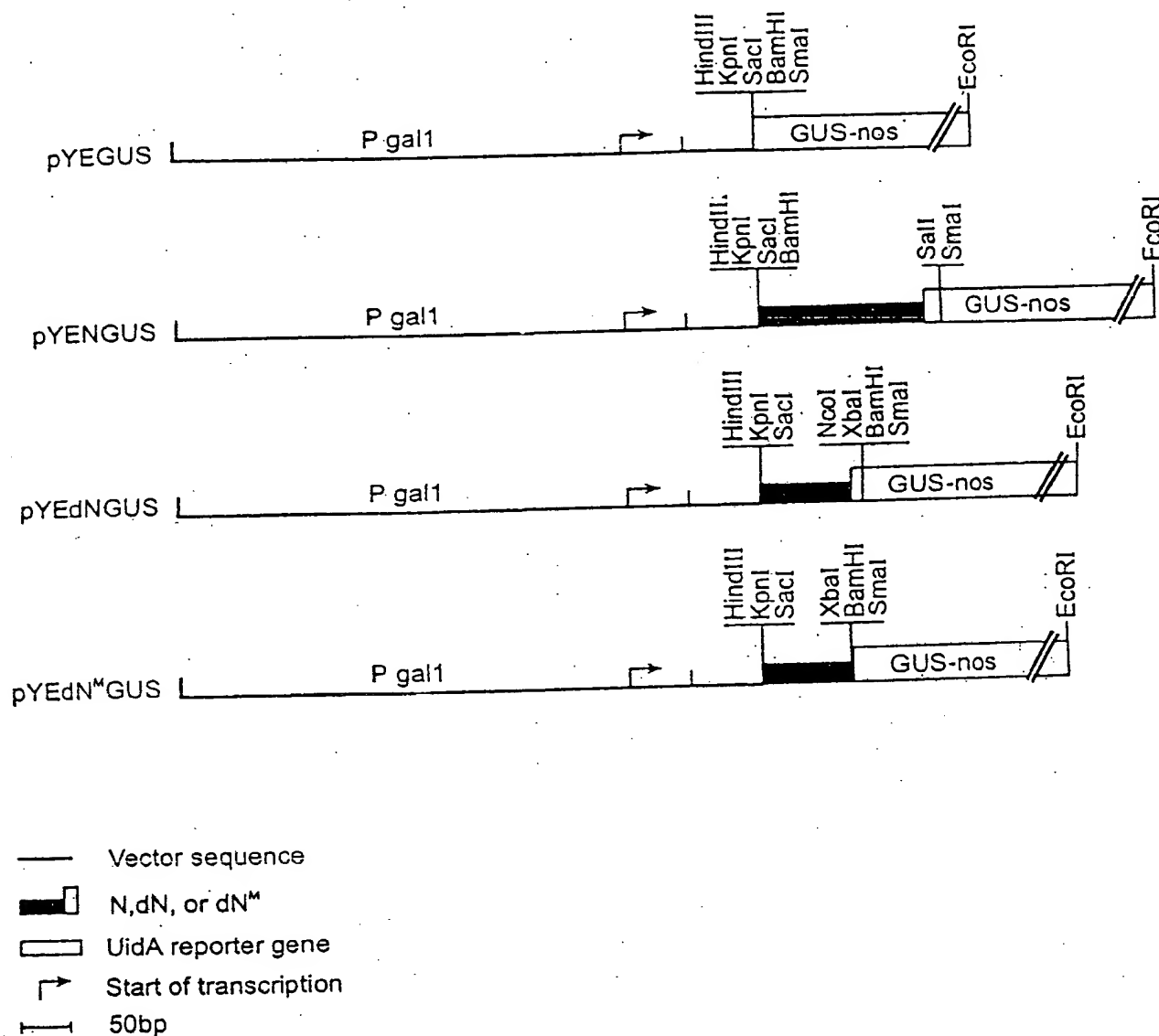


Figure 5A



Title: TRANSLATIONAL REGULATORY ELEMENTS

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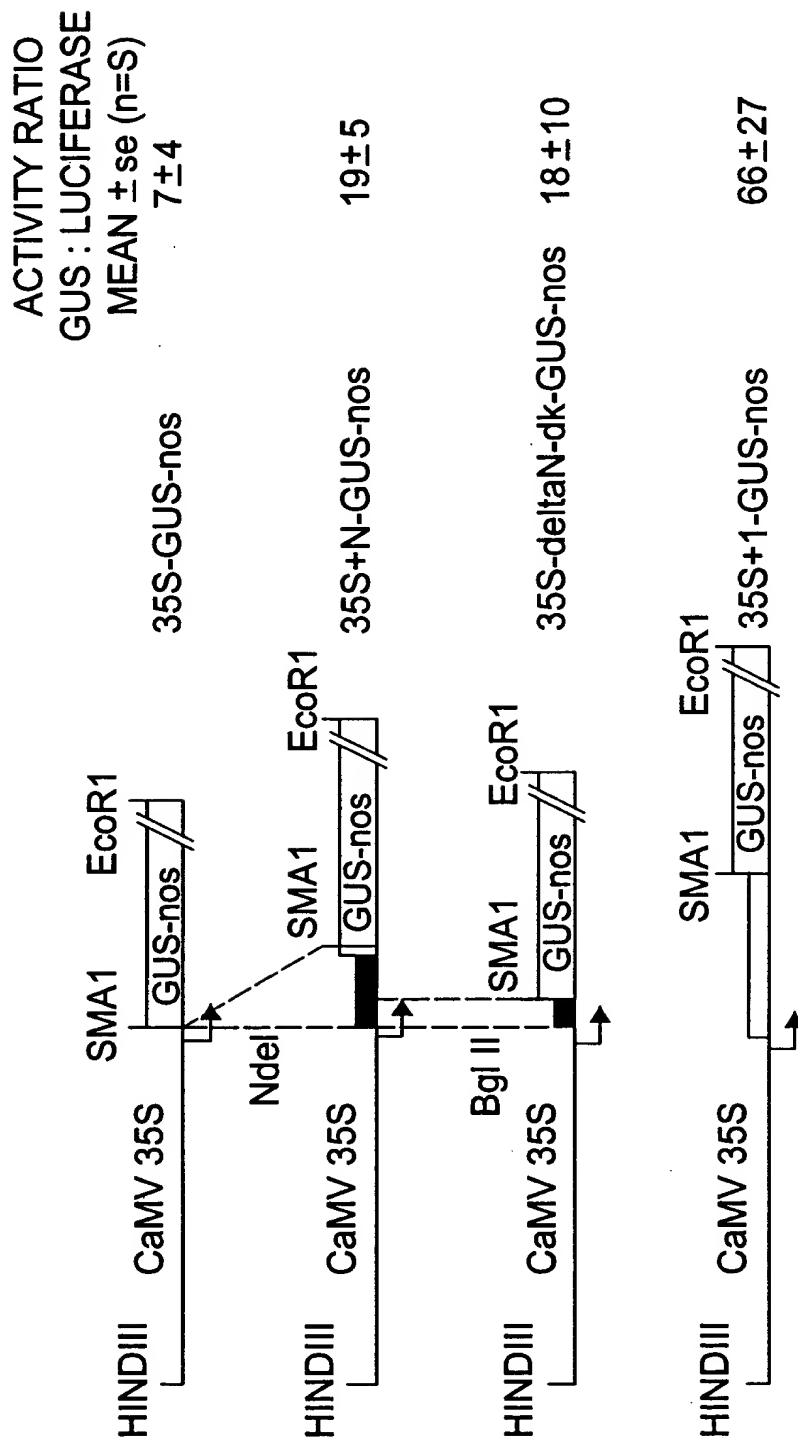


FIG. 5B

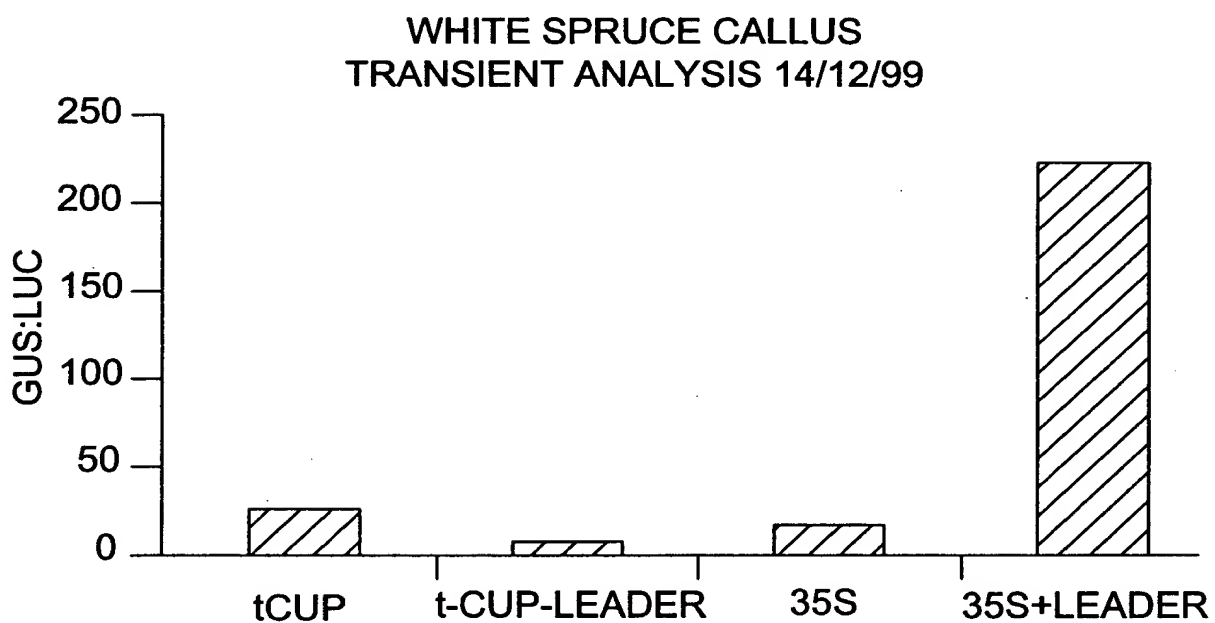


Title: TRANSLATIONAL REGULATORY ELEMENTS

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CONSTRUCT

FIG. 5C



Title: TRANSLATIONAL REGULATORY ELEMENTS

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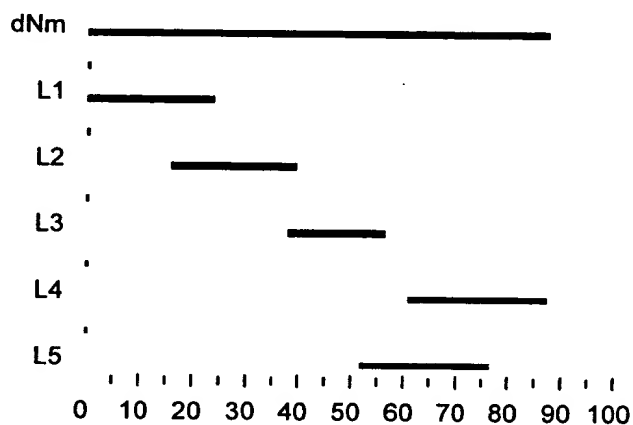


Figure 6A

Linker 1: GGATCTATCCTCTTATCTCTCAA

Linker 2: ATCTCTCAAACCTCTCTCGAACCTT

Linker 3: TTCCCCTAACCCTAGCAG

Linker 4: ATCATCCTCACCTCAAAACCCACC

Linker 5: AGCCTCTCATCATCCTCACCTCAA

Figure 6B



Title: TRANSLATIONAL REGULATORY ELEMENTS

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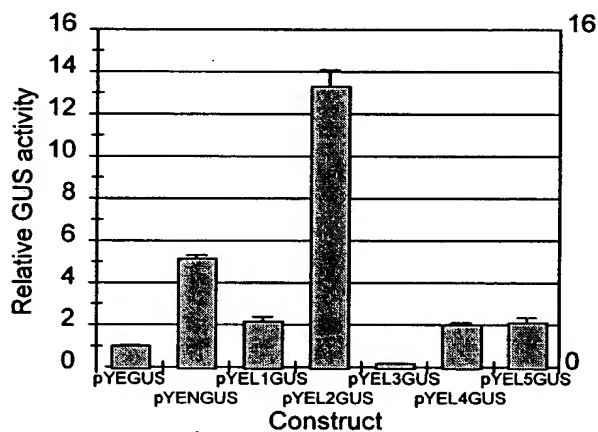


Figure 6C

L2	AUCUCUCAA ACUCUCUC GAACCUU
L2C	AUCUCUCAAACUCUCU
L2R	ACUCUCUCGAACCUU

Figure 6D



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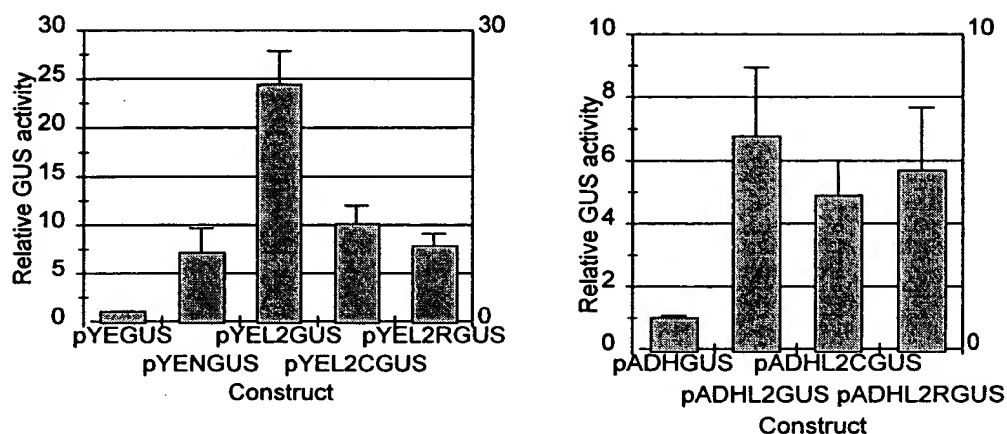


Figure 6E

L2	A	TCT	CTC	AAA	CTC	TCT	CGA	ACC	TT
SCAN1	a	AGA	ctc	aaa	ctc	tct	cga	acc	tt
SCAN2	a	tct	GAG	aaa	ctc	tct	cga	acc	tt
SCAN3	a	tct	ctc	GGG	ctc	tct	cga	acc	tt
SCAN4	a	tct	ctc	aaa	GAG	tct	cga	acc	tt
SCAN5	a	tct	ctc	aaa	ctc	AGA	cga	acc	tt
SCAN6	a	tct	ctc	aaa	ctc	tct	GCT	acc	tt
SCAN7	a	tct	ctc	aaa	ctc	tct	cga	GAG	tt

Figure 6F



Title: TRANSLATIONAL REGULATORY ELEMENTS

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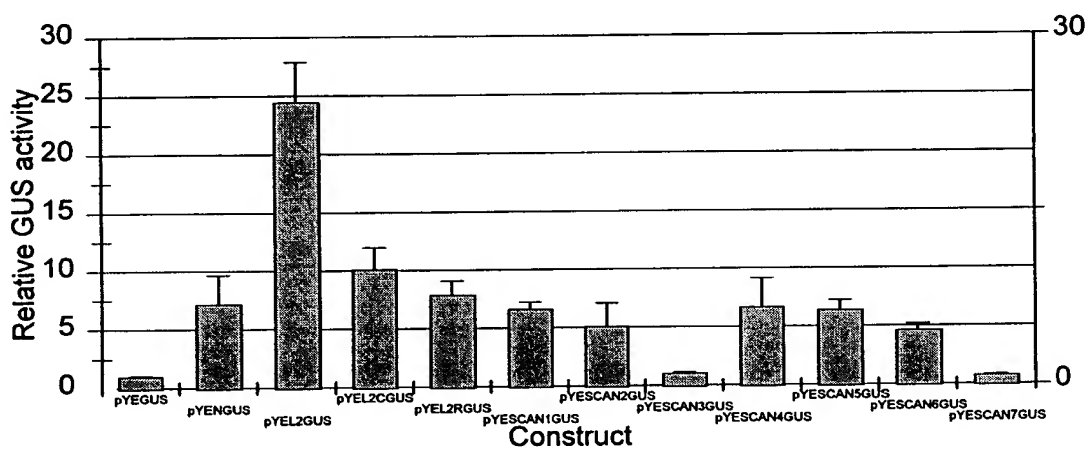
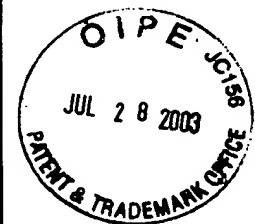


Figure 6G



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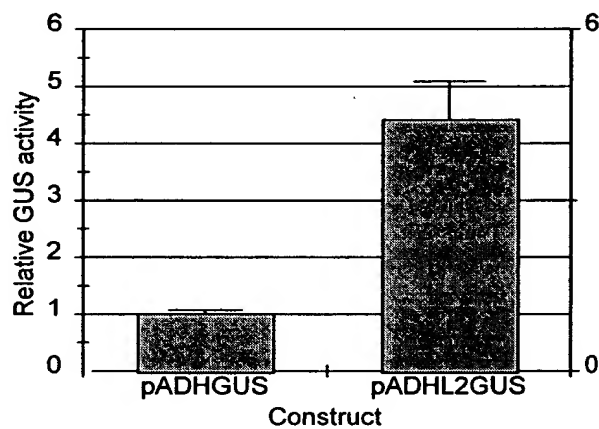


Figure 6H

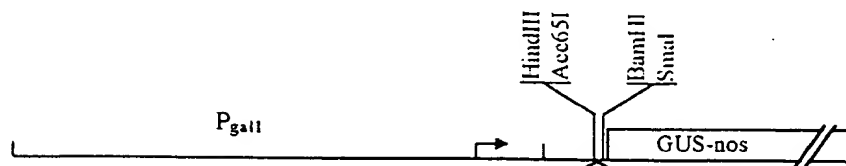


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pYEGUS	(no sequence)
pYEL1GUS	GGATCTATCCTCTTATCTCTCAA
pYEL2GUS	ATCTCTCAAACCTCTCTCGAACCTT
pYEL3GUS	TTCCCCTAACCCCTAGCAG
pYEL4GUS	ATCATCCTCACCTCAAAACCCACC
pYEL5GUS	AGCCTCTCATCATCCTCACCTCAA
pYEL2CGUS	ATCTCTCAAACCTCTCT
pYEL2RGUS	ACTCTCTCGAACCTT
pYELMGUS	ACTCTCTC
pYESCAN1GUS	AAGACTCAAACCTCTCTCGAACCTT
pYESCAN2GUS	ATCTGAGAACTCTCTCGAACCTT
pYESCAN3GUS	ATCTCTCGGGCTCTCTCGAACCTT
pYESCAN4GUS	ATCTCTCAAAGAGTCTCGAACCTT
pYESCAN5GUS	ATCTCTCAAACCTCAGACGAACCTT
pYESCAN6GUS	ATCTCTCAAACCTCTCTGCTACCTT
pYESCAN7GUS	ATCTCTCAAACCTCTCTCGAGAGTT
pYEB1-L2GUS	ATCTCTCAAACCTATCTCGAACTT
pYEB7-L2GUS	ATCTCTCAAACCTCTCTCAAACCTT
pYEL2D1GUS	ATCTCTC---CTCTCTCGAACCTT
pYEL2D2GUS	ATCTCTCAAACCTCTCTCGA---TT
pYEL2D3GUS	ATCTCTC---CTCTCTCGA---TT
pYE2L2GUS	ATCTCTCAAACCTCTCTCGAACCTTTCTCTCAAACCTCTCTCGAACCTT

LEGEND:

- Vector sequence
- GUS reporter gene
- Start of transcription

FIGURE 6I



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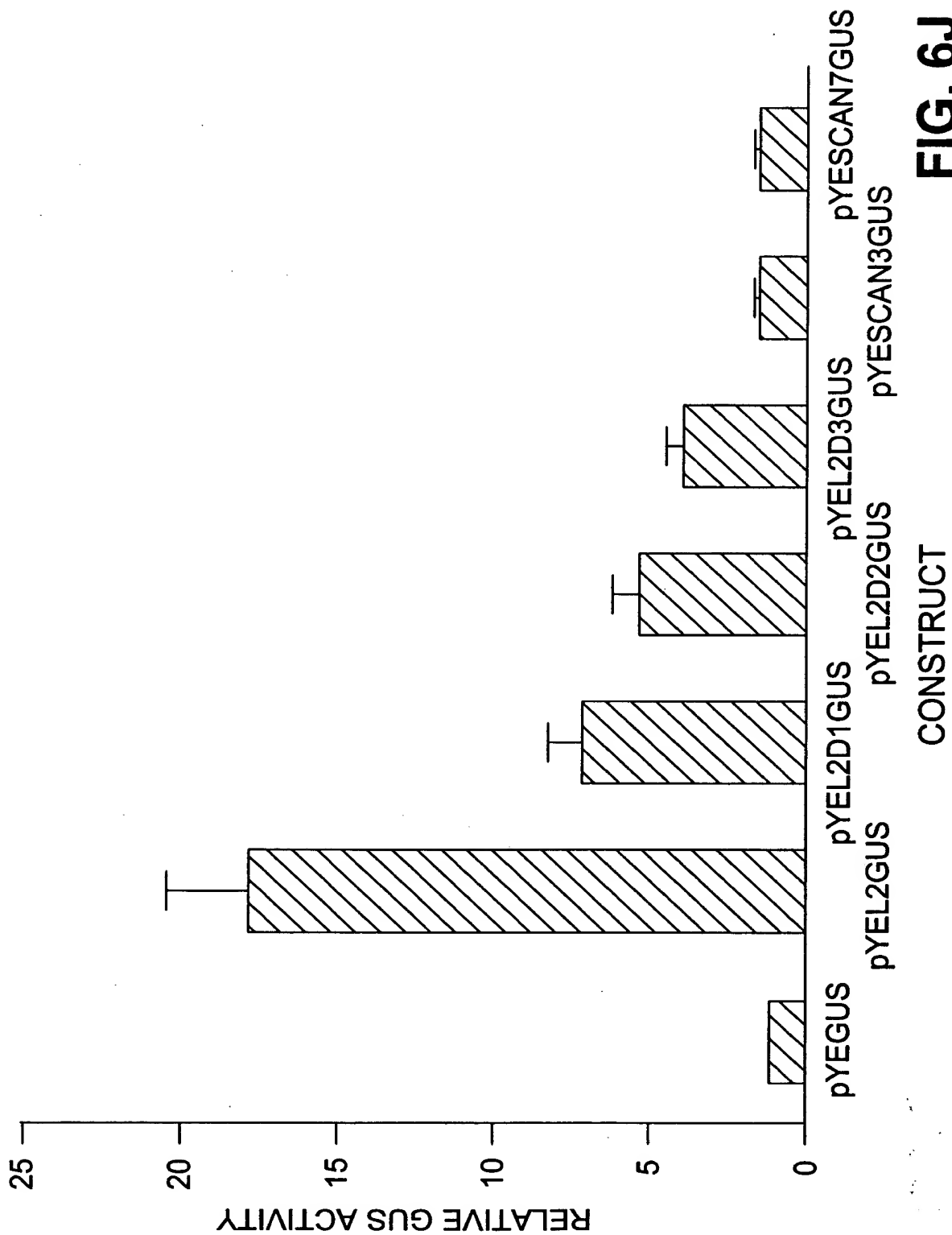


FIG. 6J



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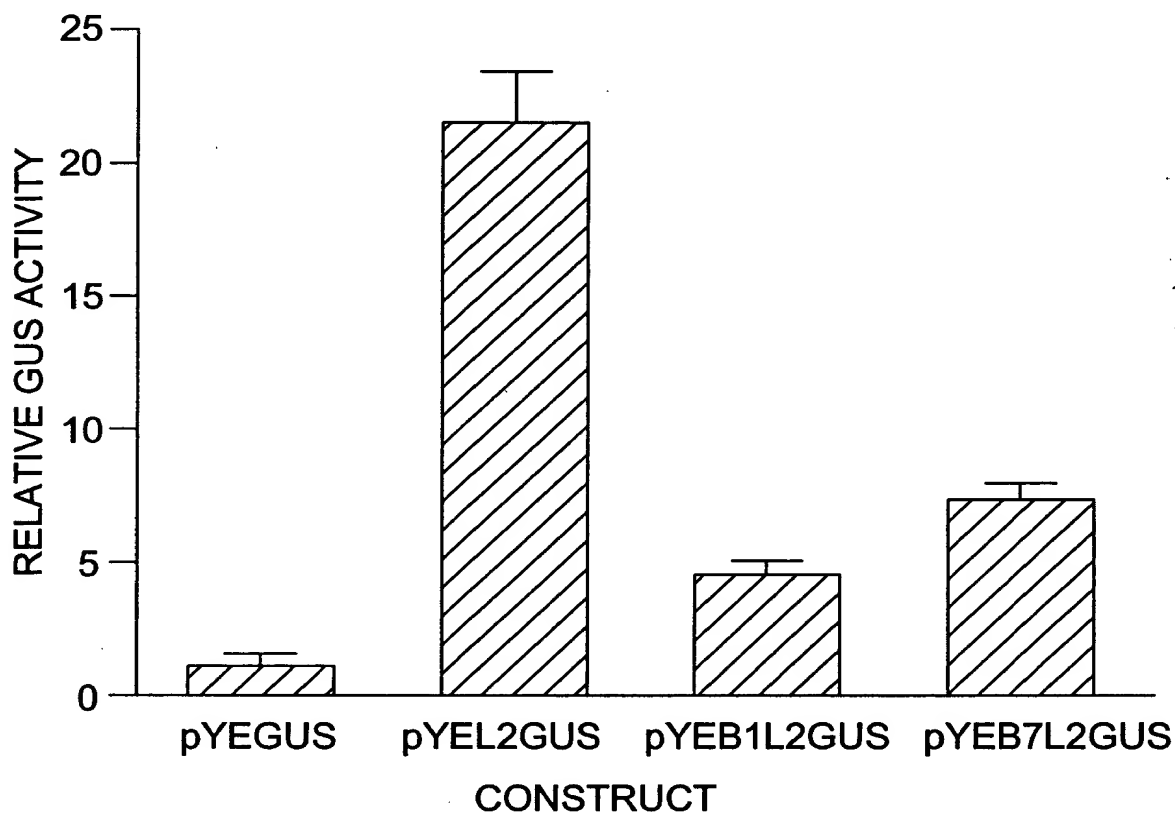


FIG. 6K



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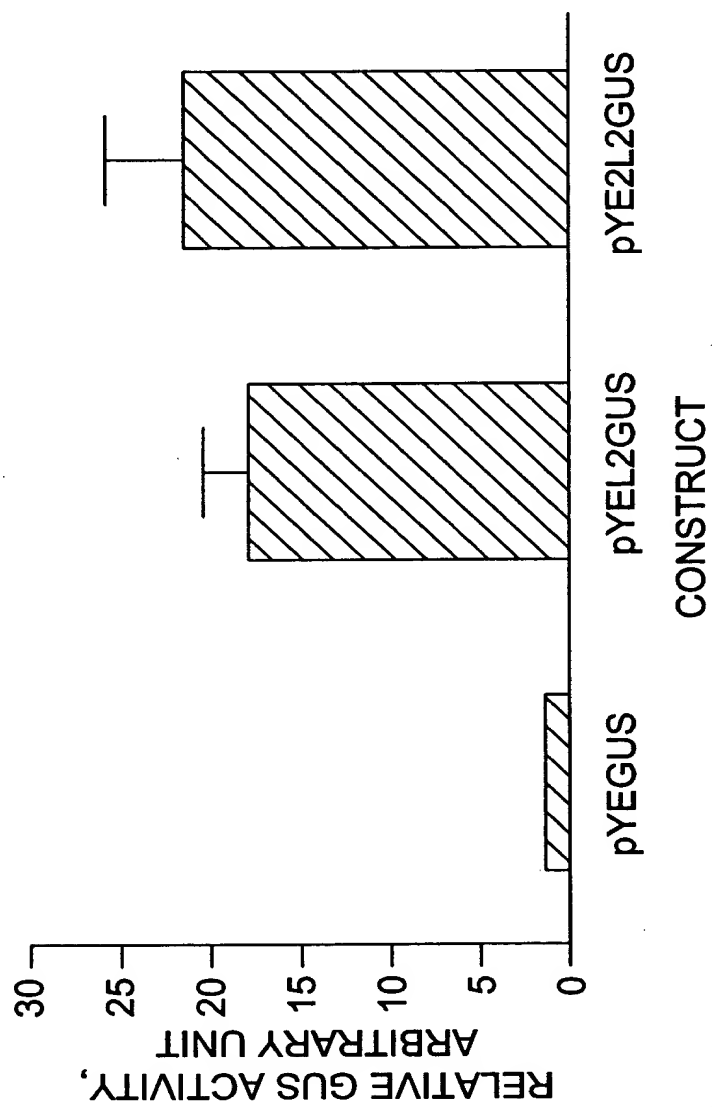


FIG. 6L

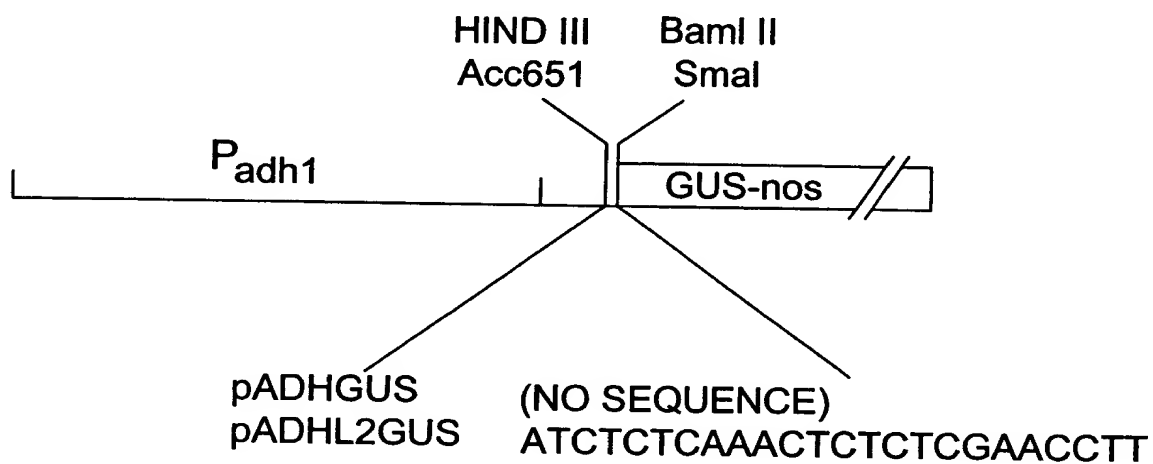


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LEGEND:

— VECTOR SEQUENCE

□ GUS REPORTER GENE

FIG. 6M



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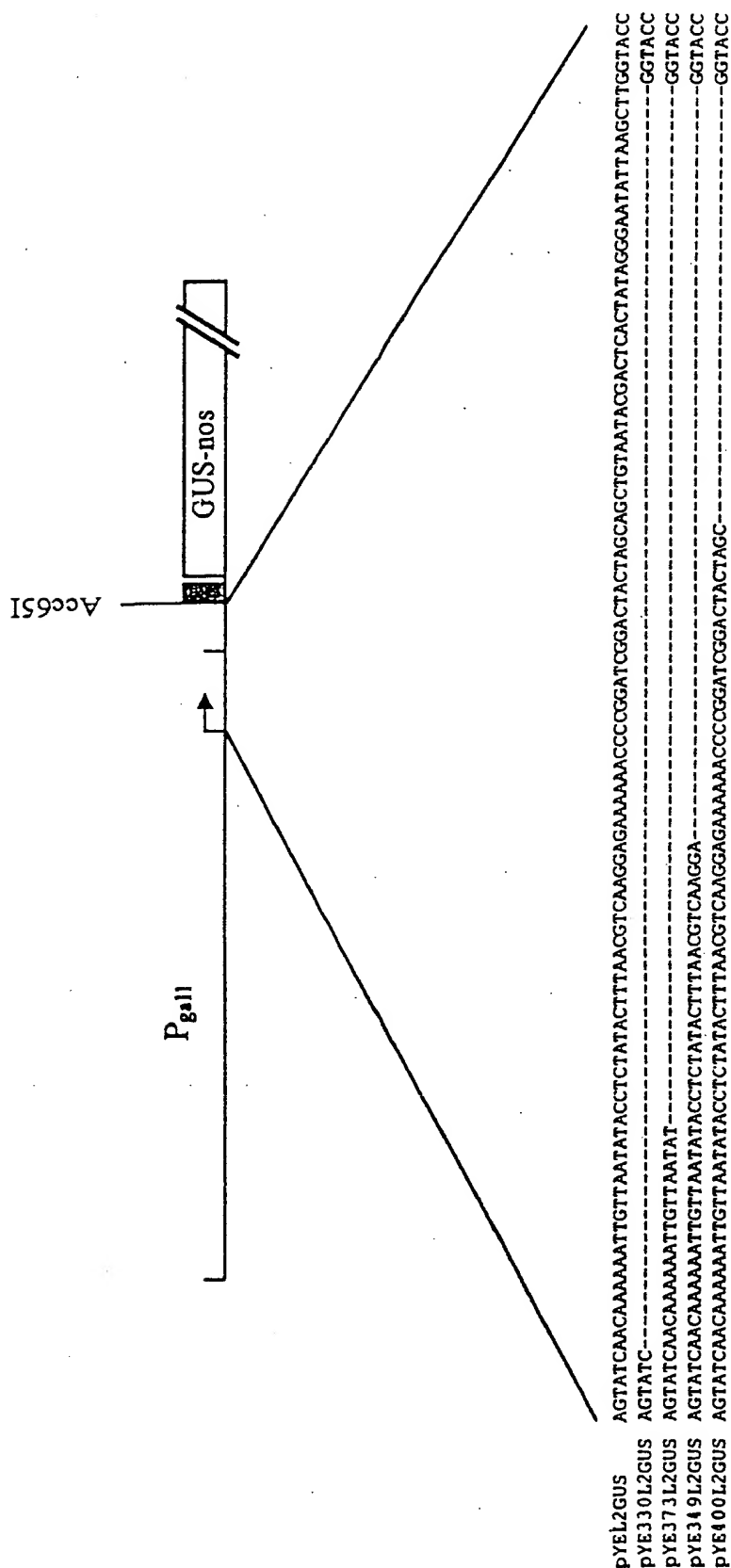


FIGURE 6N



Title: TRANSLATIONAL REGULATORY ELEMENTS

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ANALYSIS OF GUS ACTIVITY IN YEAST EXPRESSION SYSTEM

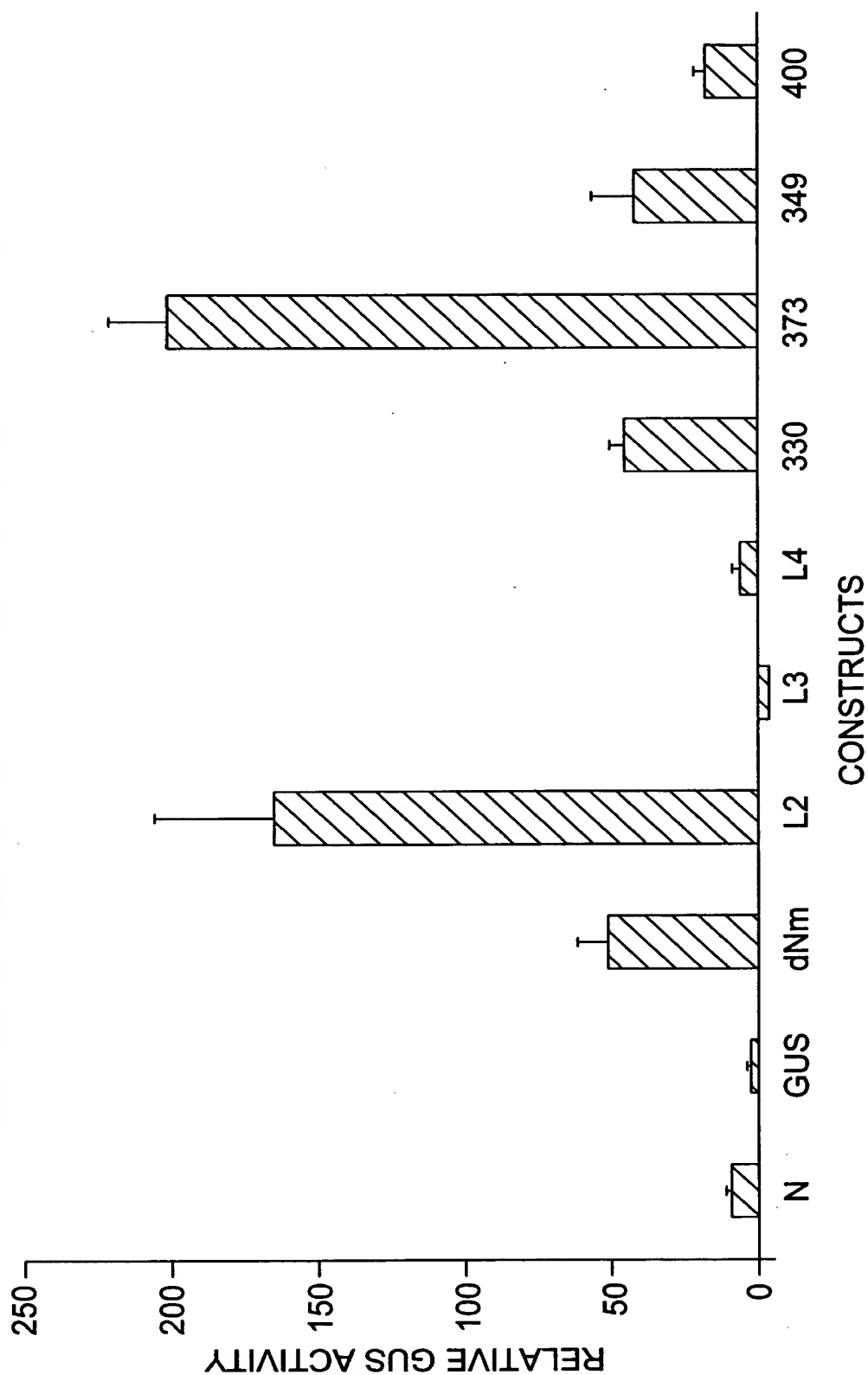


FIG. 60



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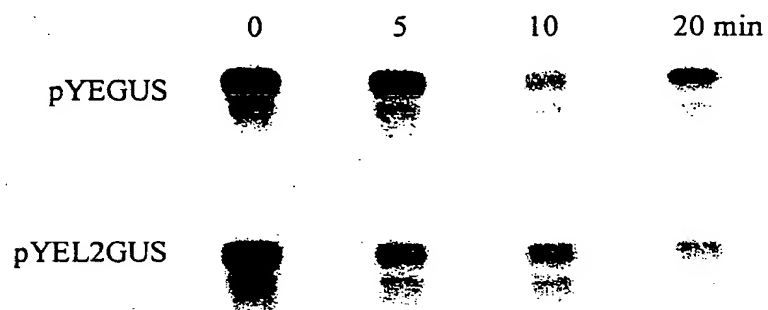


FIGURE 6P.1



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Application No.: 09/747,007

Docket Number: 411044.90030

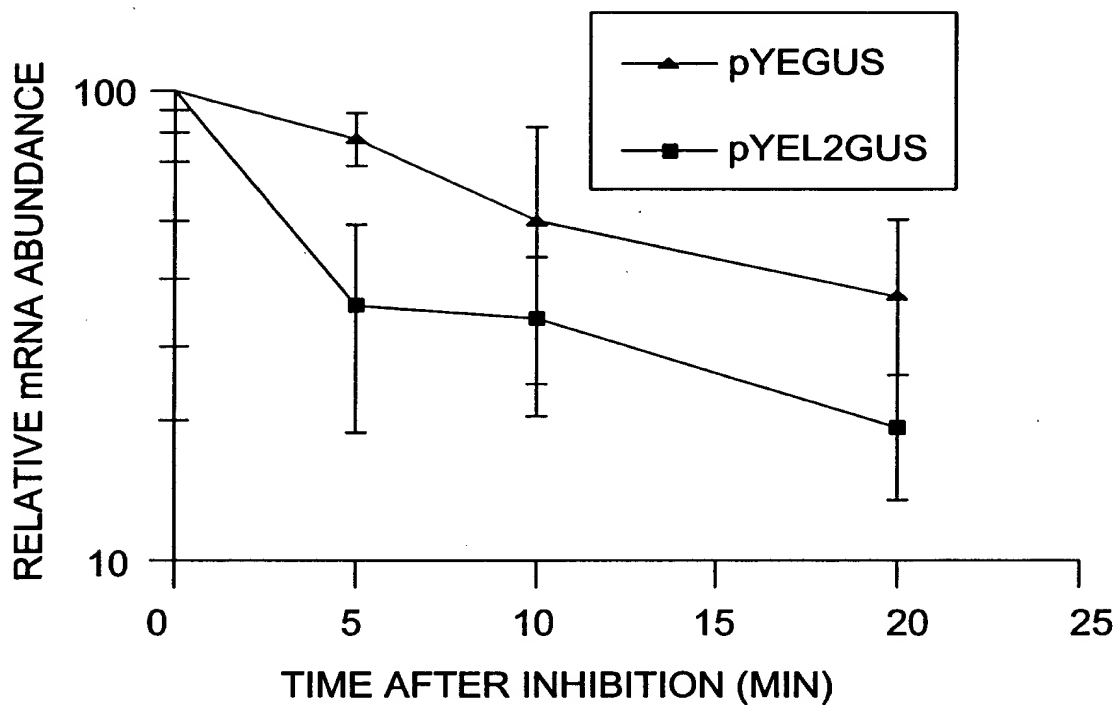


FIG. 6P.2



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

ENHANCED tCUP VERSIONS 1-3

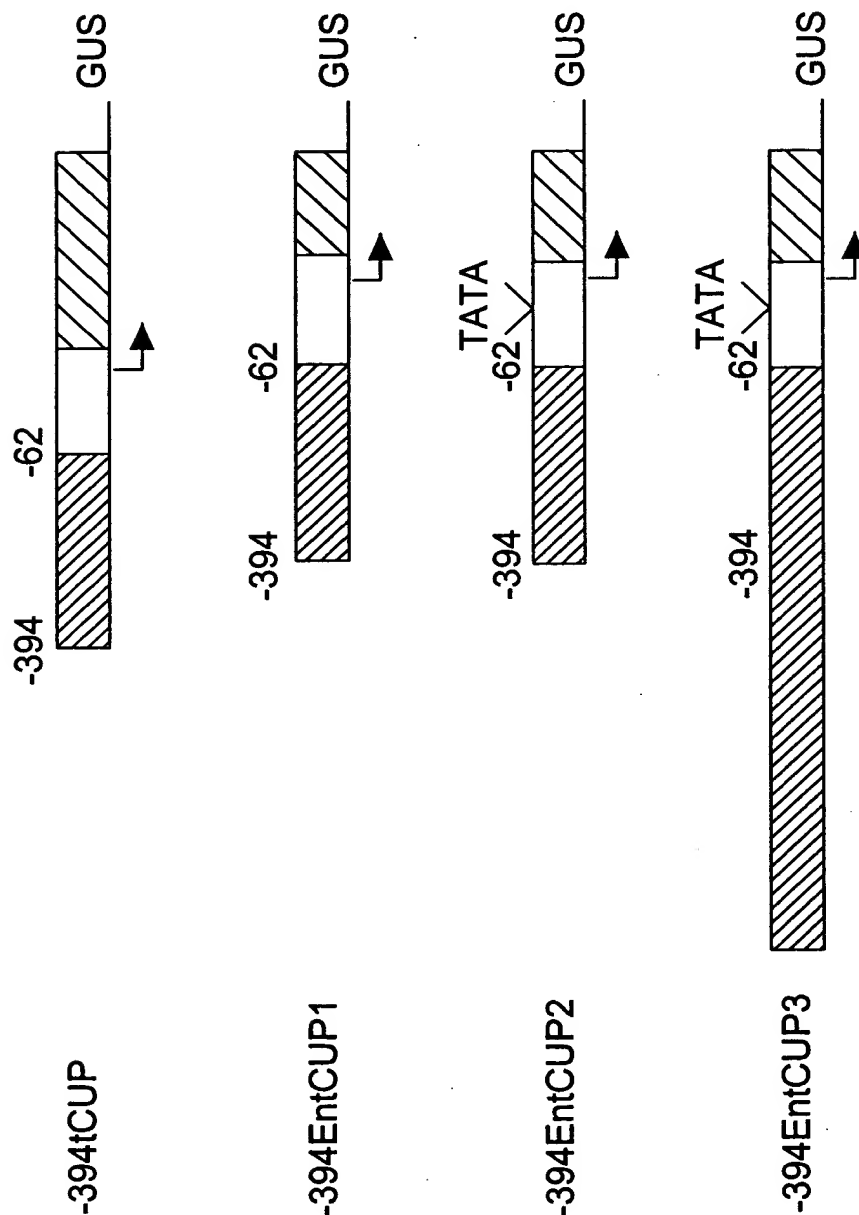


FIG. 7A.1



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

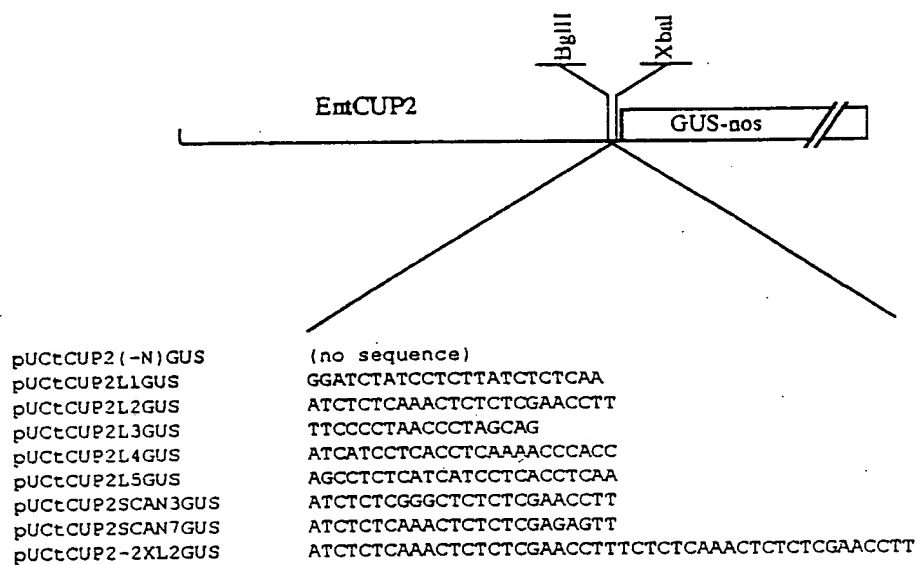


FIGURE 7A.2



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

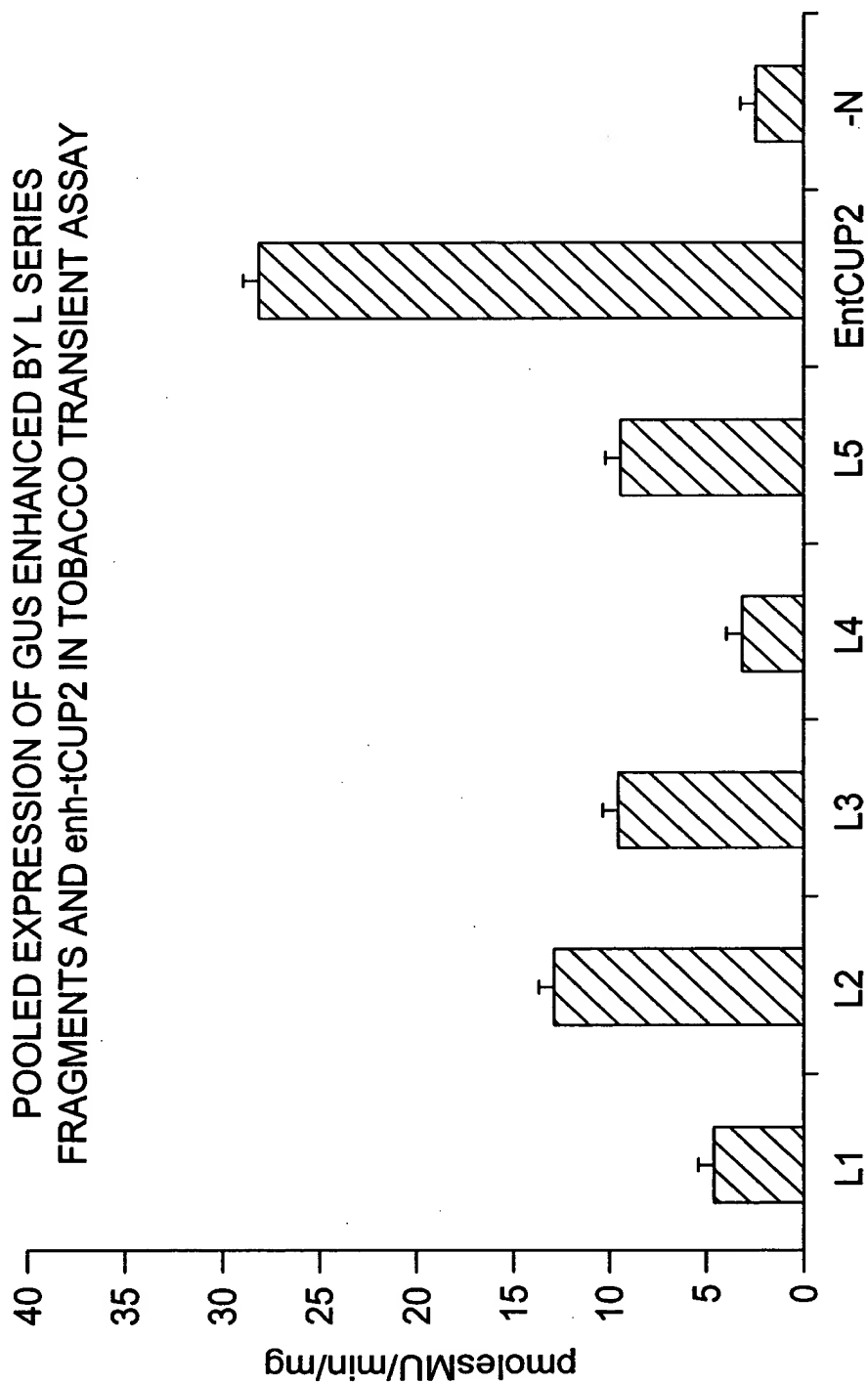


FIG. 7B



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

EVALUATION OF tCUP LEADER ELEMENT,
L1, L2, L3, L4, AND L5 ON TRANSIENT GUS GENE EXPRESSION
IN ALFALFA SUSPENSION CULTURE

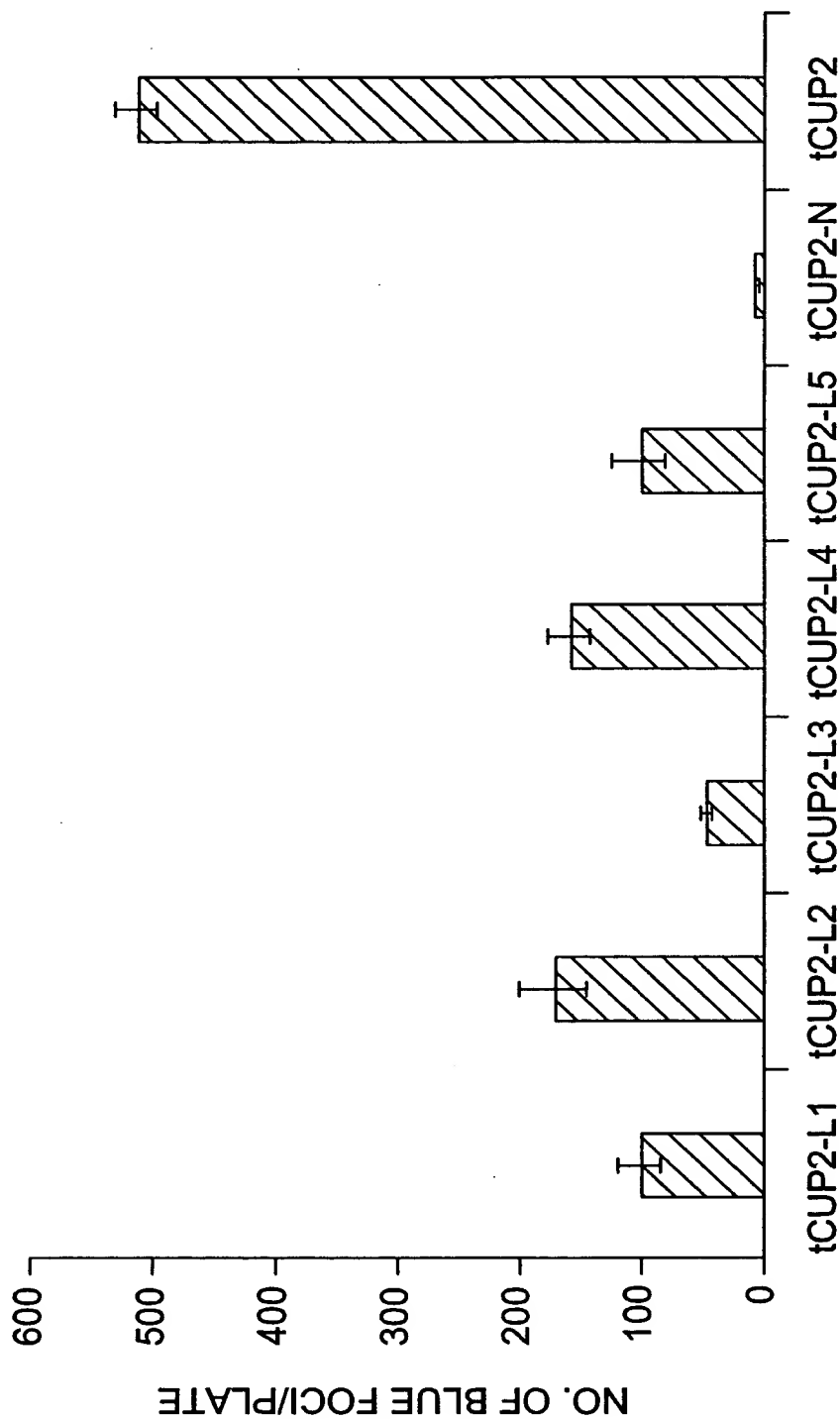


FIG. 7C



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

EVALUATION OF tCUP LEADER ELEMENTS, L1, L2, L3, L4, AND L5
TRANSIENT GUS GENE EXPRESSION IN WHITE SPRUCE CALLUS

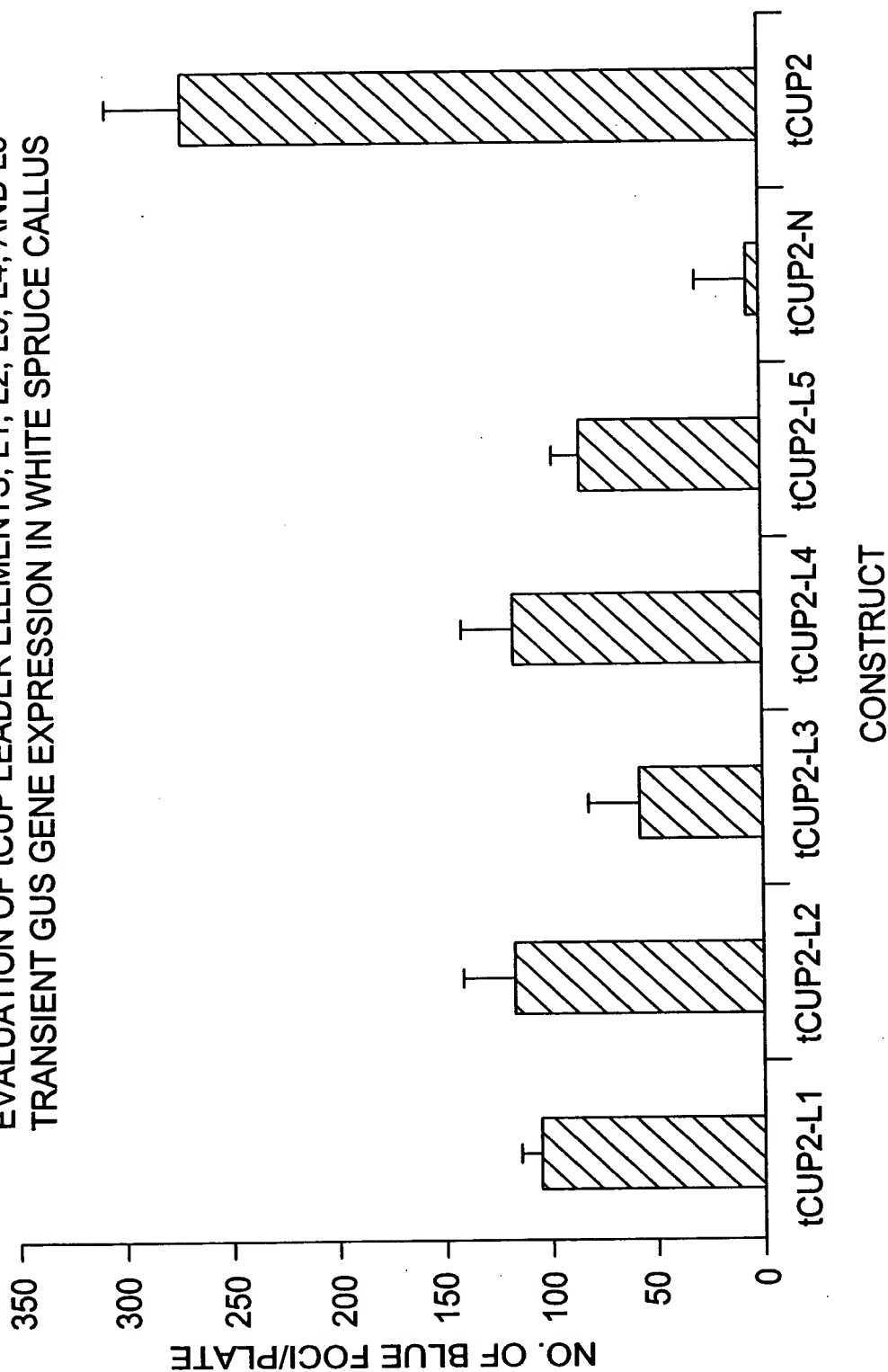


FIG. 7D

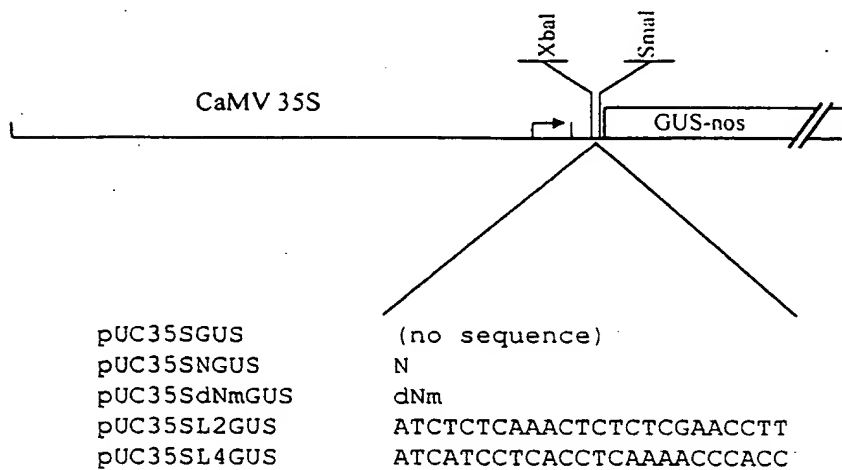


Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030



LEGEND:

- Vector sequence
- GUS reporter gene
- Start of transcription

FIGURE 8A



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

STABLE TRANSFORMATION OF *Arabidopsis* WITH
GUS ENHANCED BY L-SERIES CONSTRUCTS AND
THE 35S PROMOTER

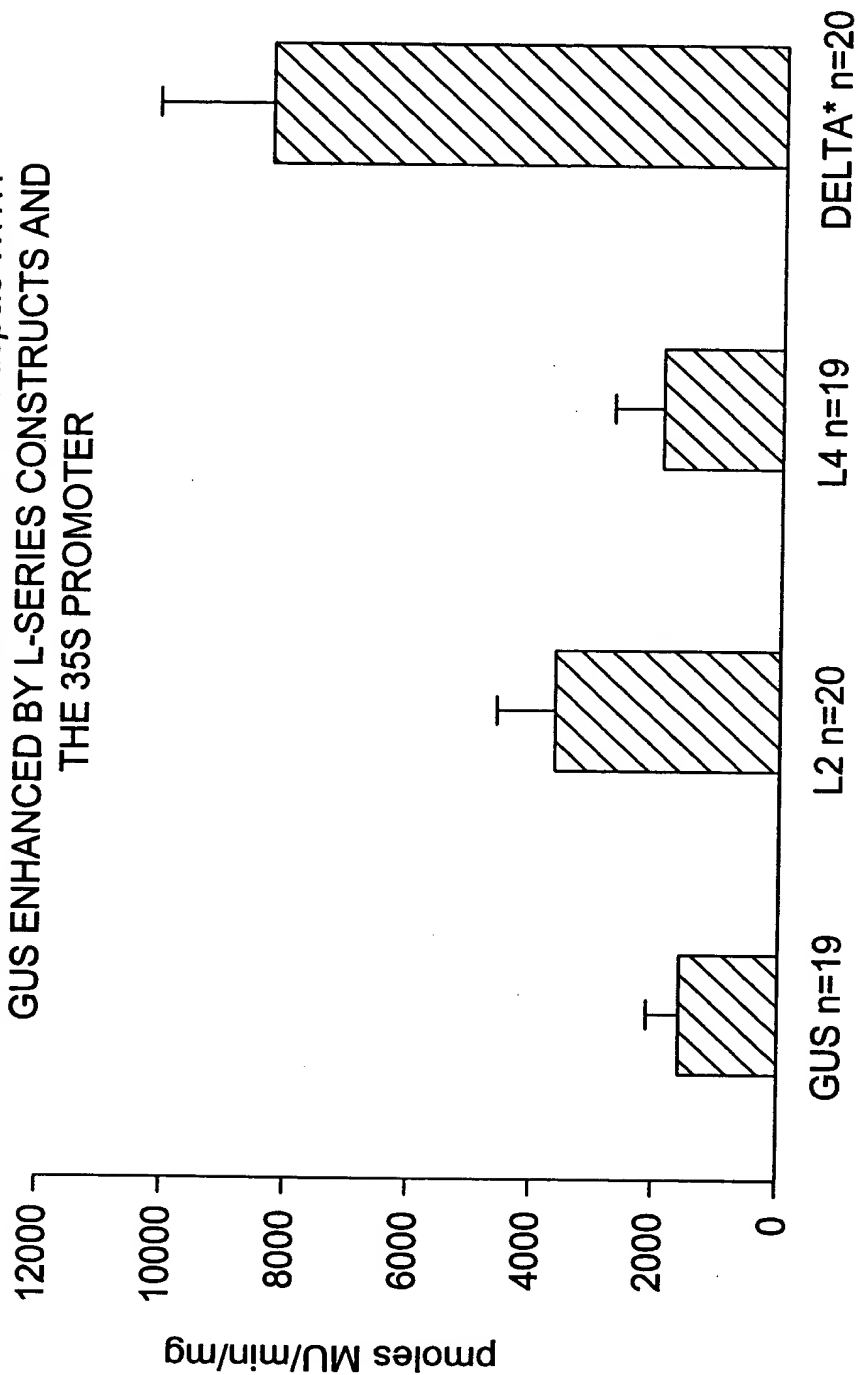


FIG. 8B



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

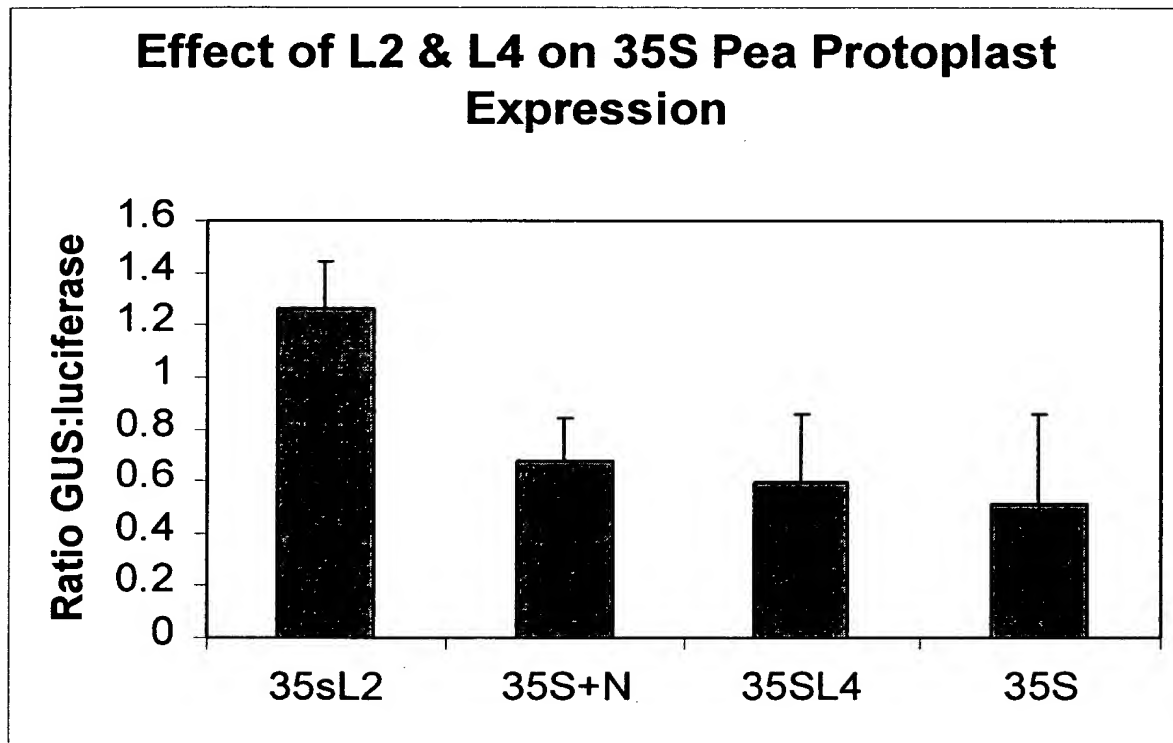


Figure 8C

Title: TRANSLATIONAL REGULATORY ELEMENTS
Inventor(s): Therese Ouellet, et al.
Application No.: 09/747,007
Docket Number: 411044.90030

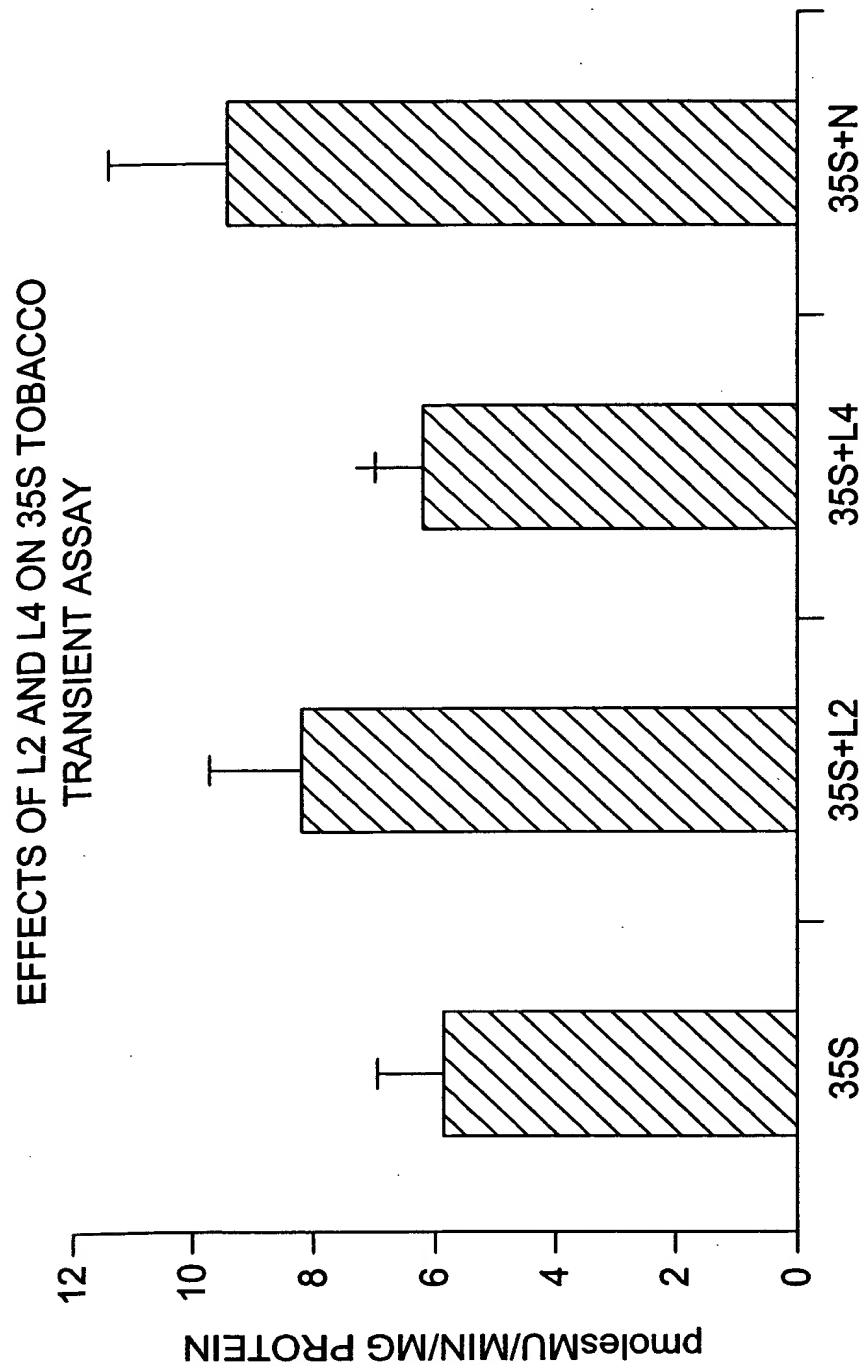


FIG. 8D

Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

EVALUATION OF THE EXPRESSION OF tCUP LEADER AND THE
ELEMENTS, L2 AND L4, WITH A HETEROGOUS PROMOTER (35S)
IN A TRANSIENT GUS GENE EXPRESSION IN
ALFALFA SUSPENSION CULTURE

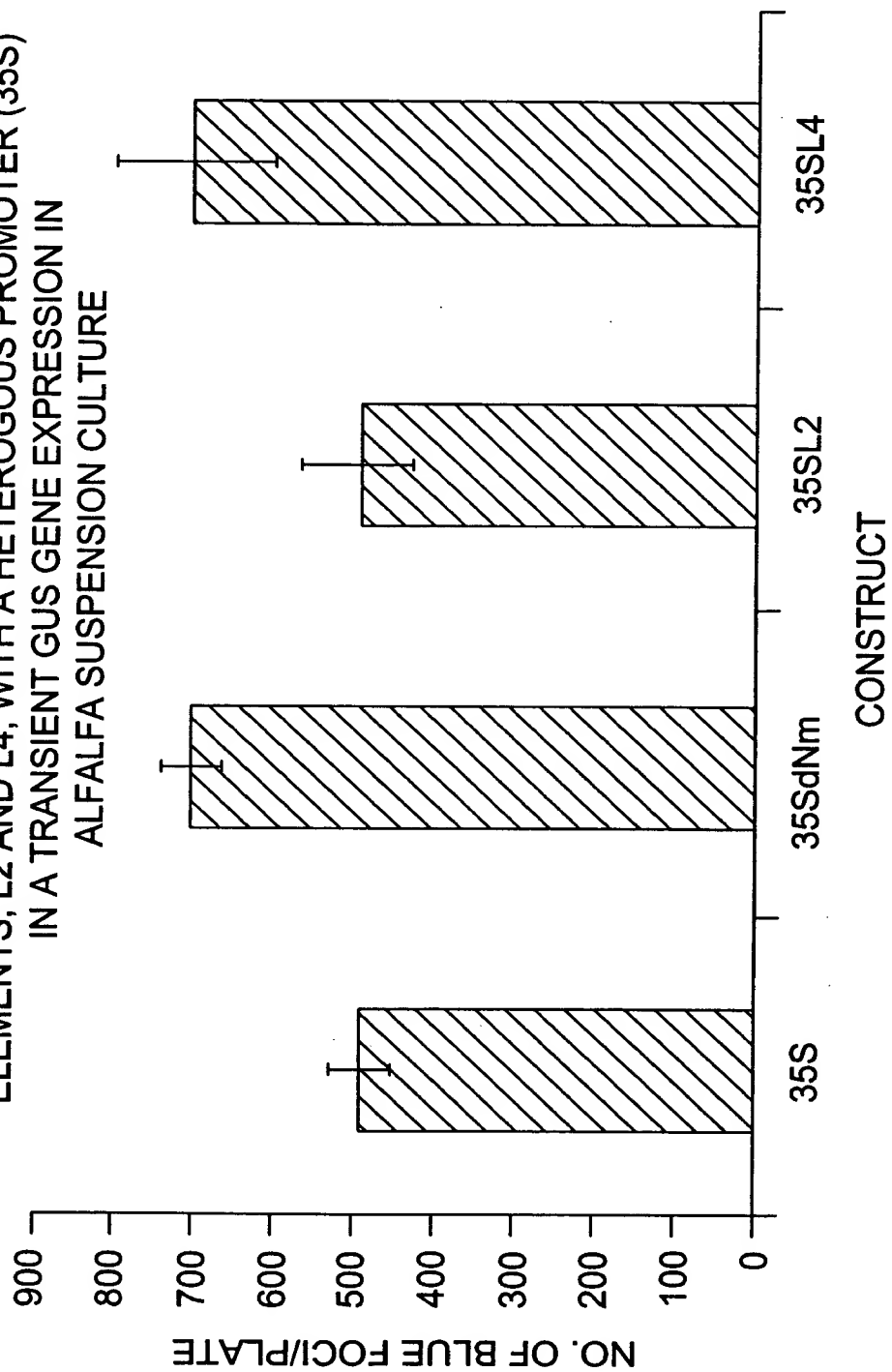


FIG. 8E



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

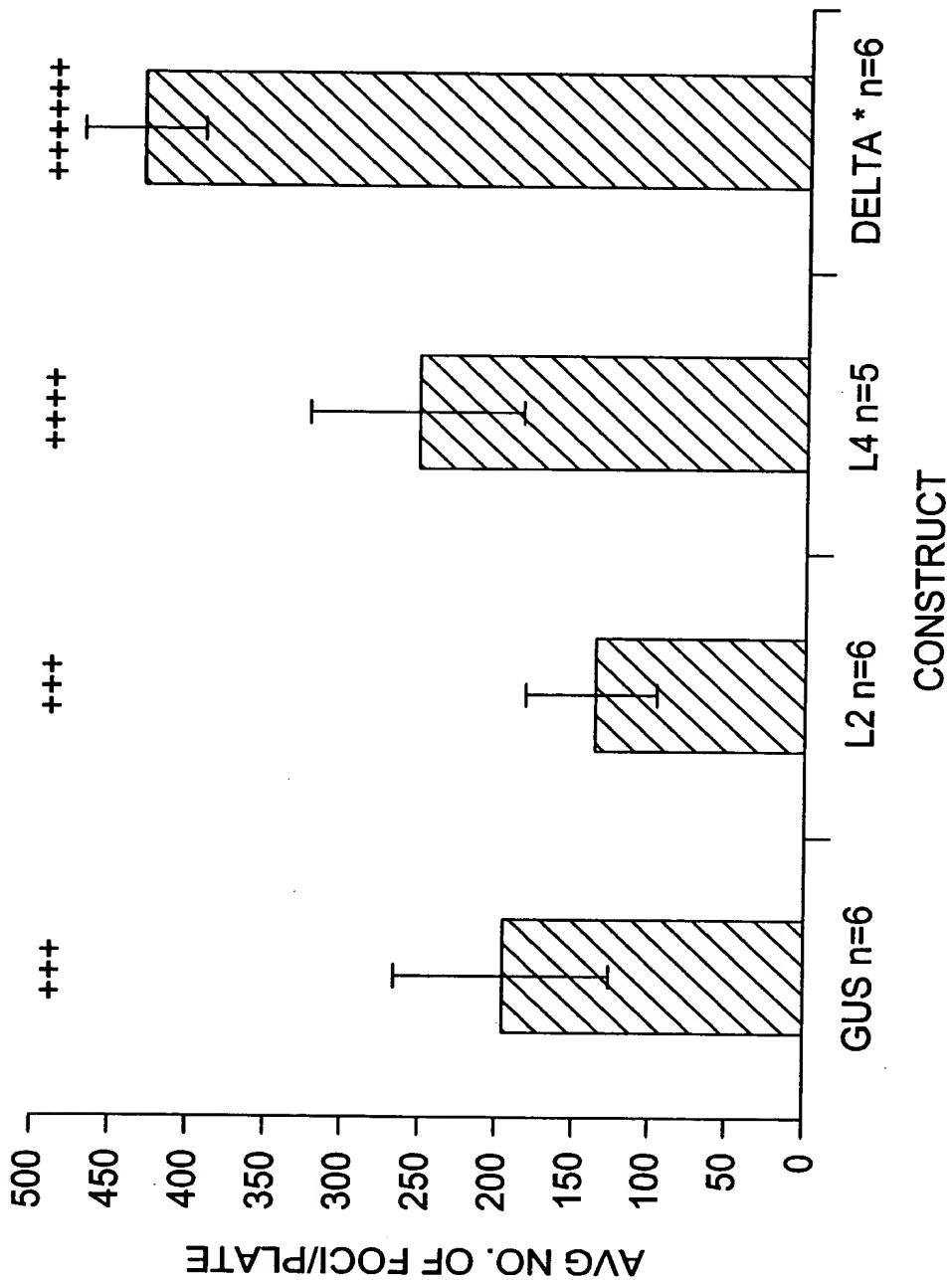


FIG. 8F

Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

EVALUATION OF THE EXPRESSION OF tCUP LEADER AND
THE ELEMENTS, L2 AND L4, WITH A HETEROGEOUS
PROMOTER (35S) IN A TRANSIENT GUS GENE EXPRESSION
IN WHITE SPRUCE CALLUS

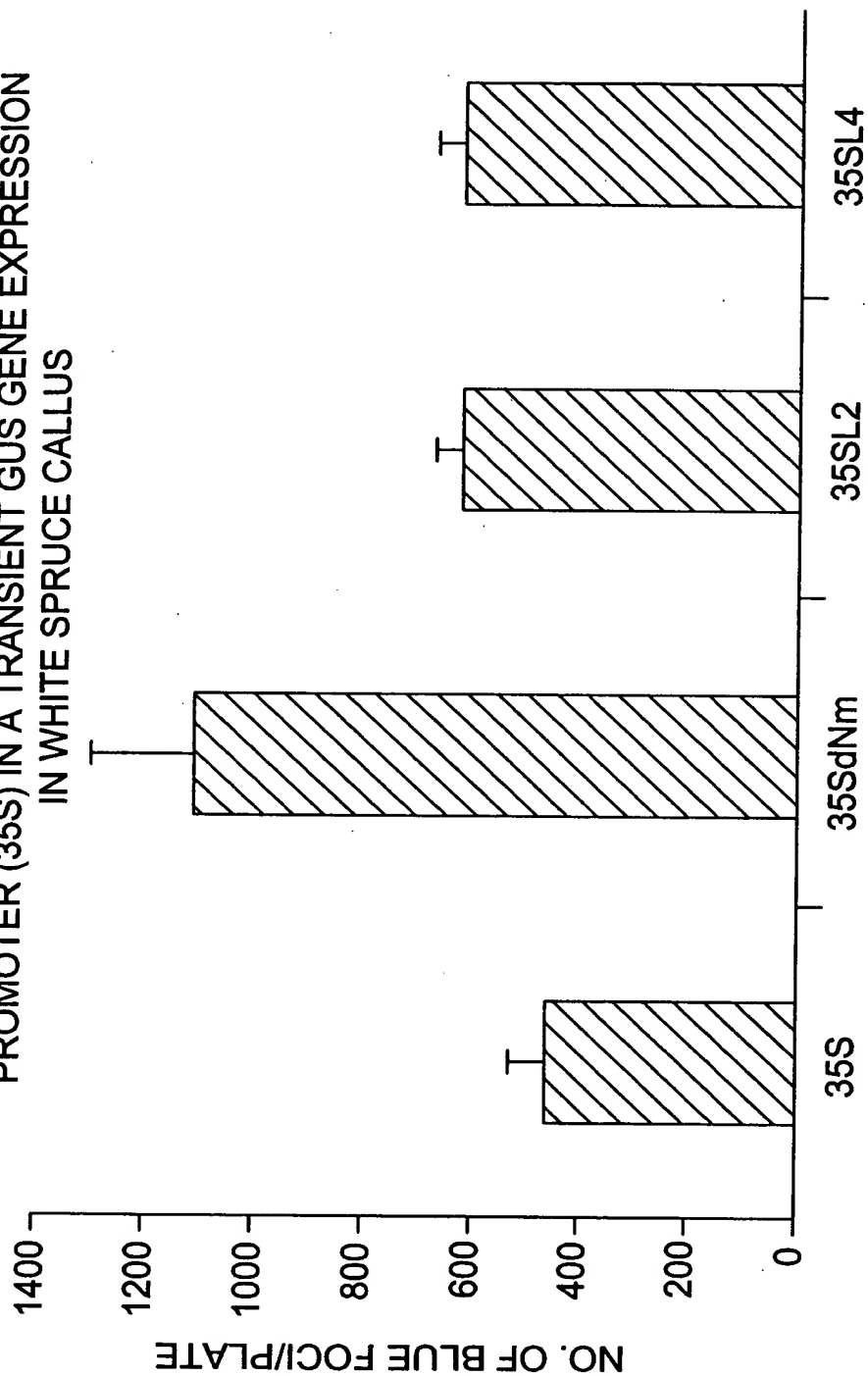


FIG. 8G

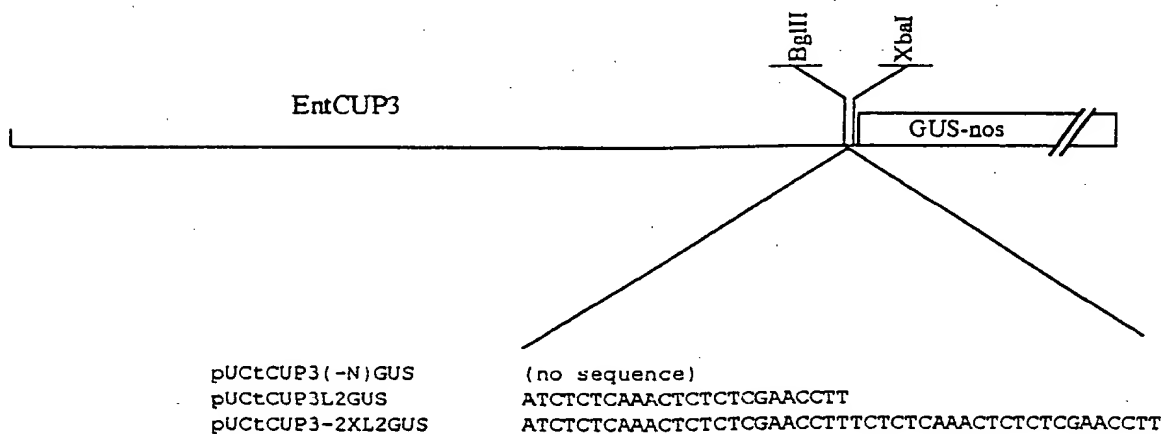


Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030



LEGEND:

- Vector sequence
- GUS reporter gene

FIGURE 9A



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

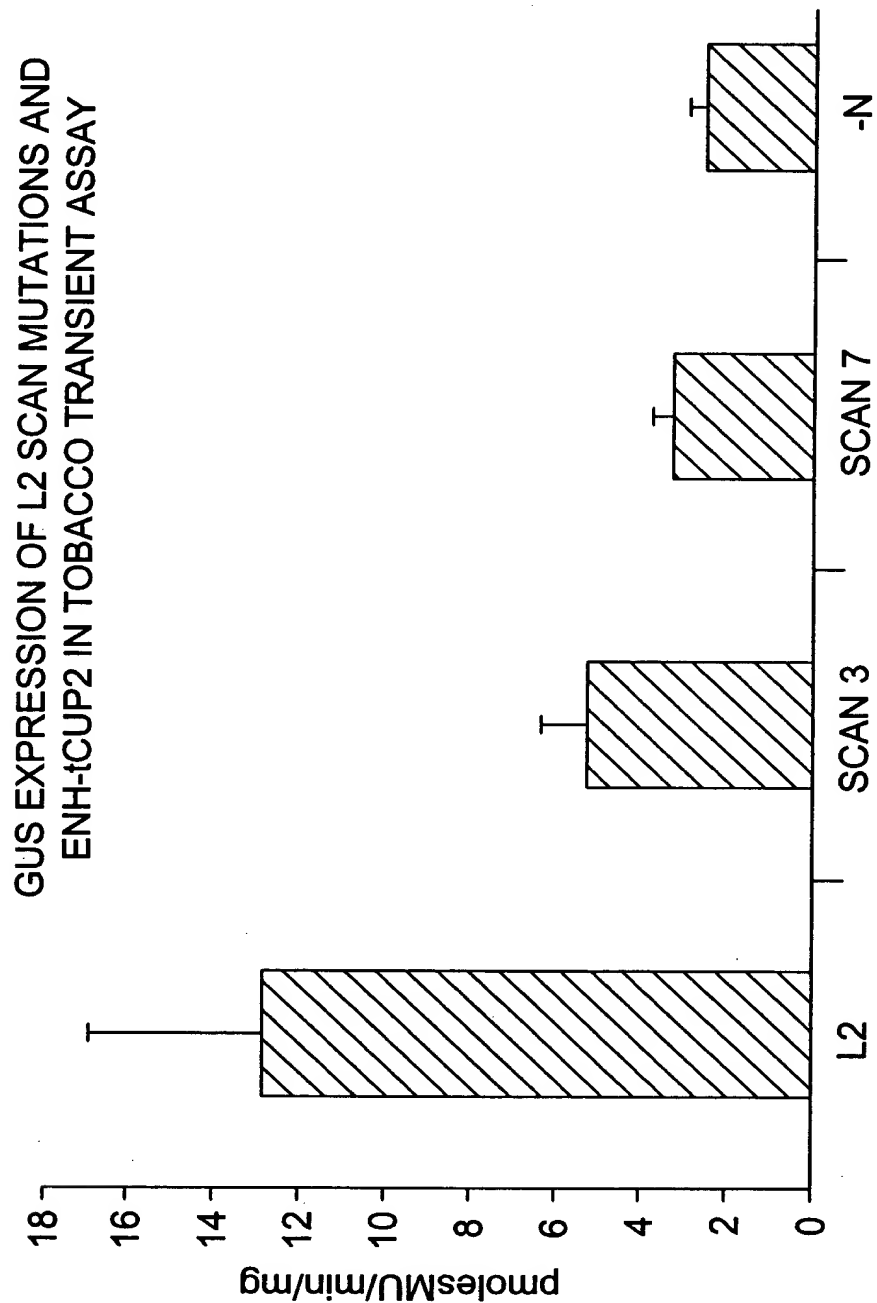


FIG. 9B



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

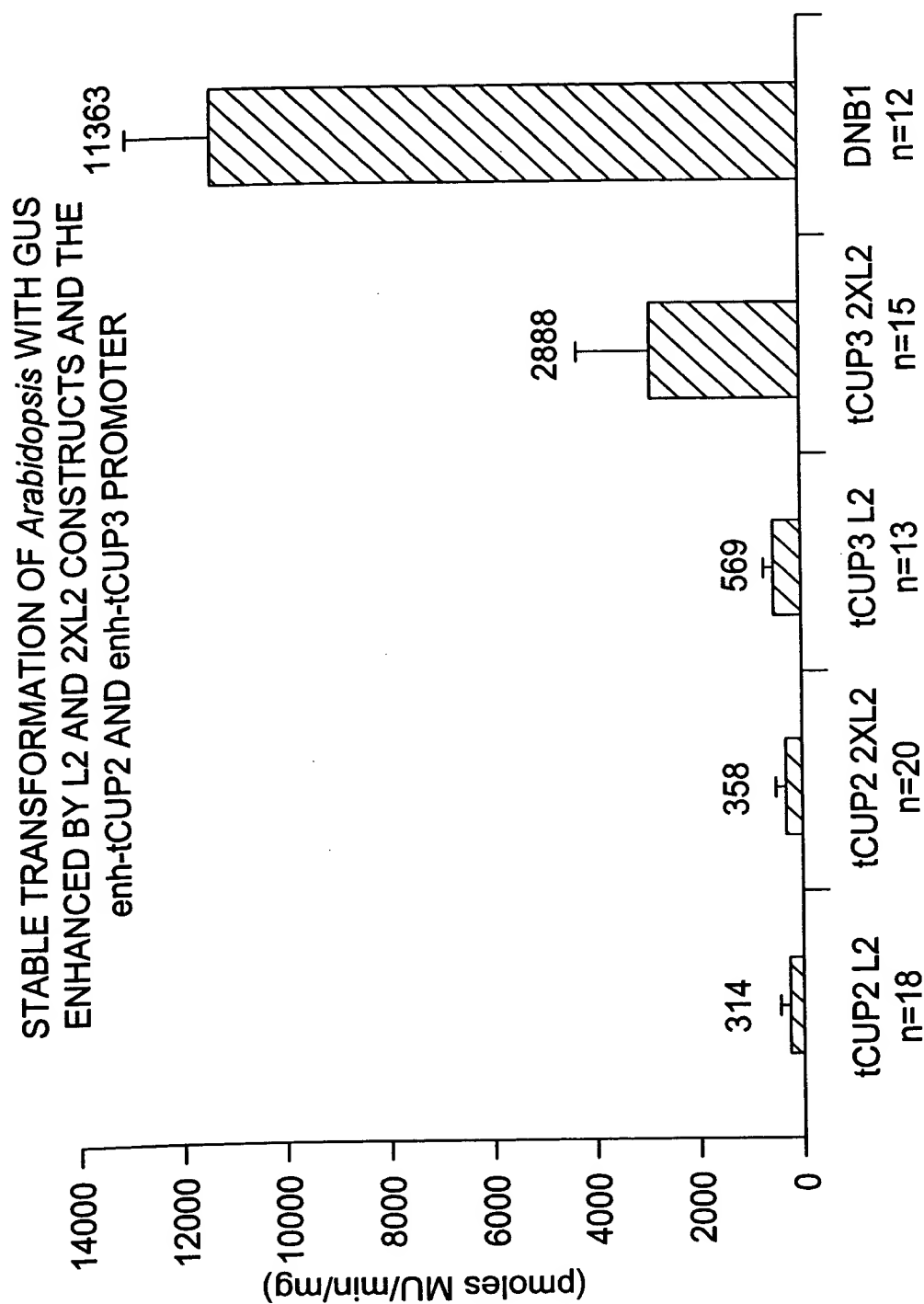


FIG. 9C



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

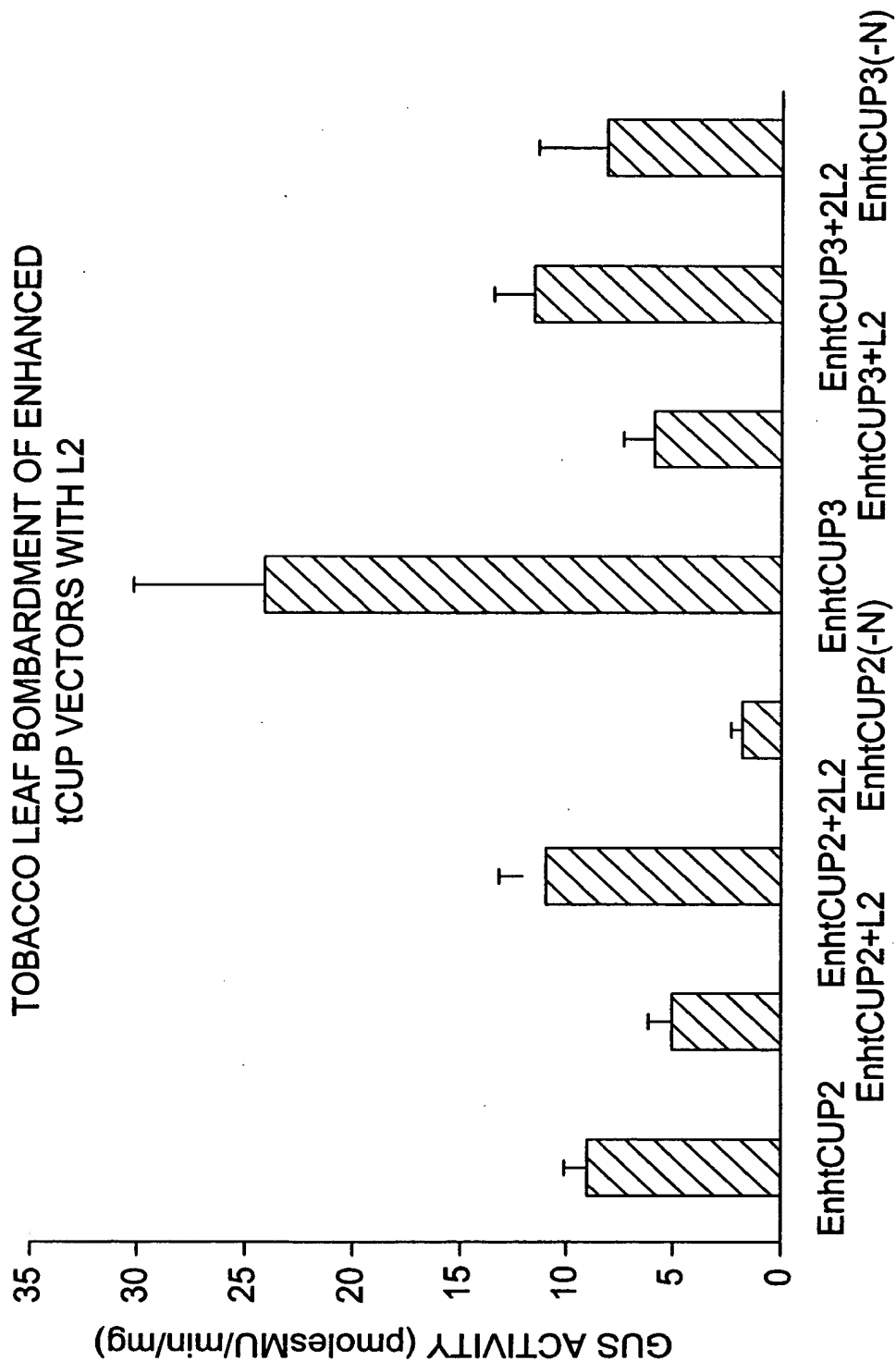


FIG. 9D

JUL 28 2003



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

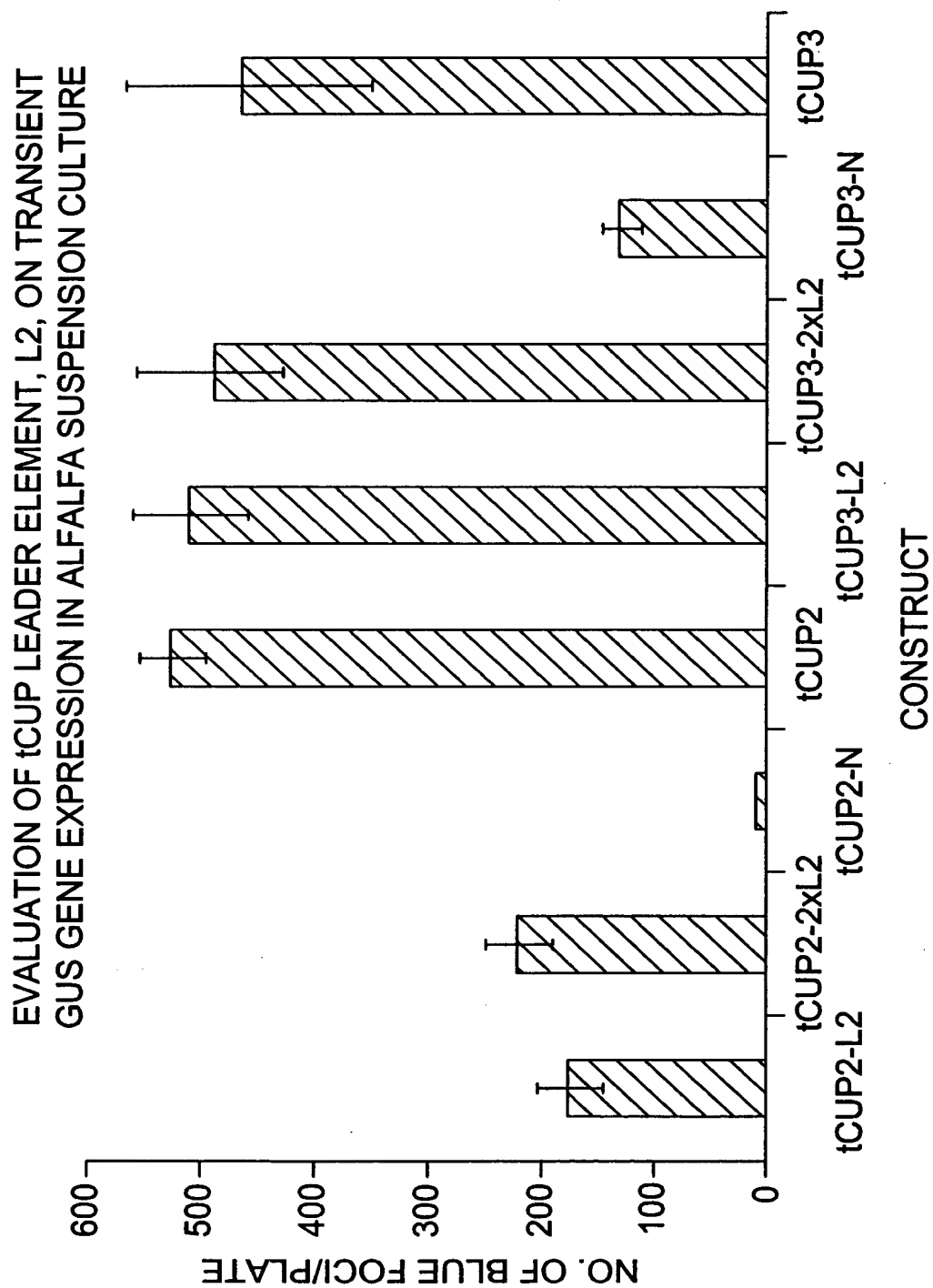


FIG. 9E



Title: TRANSLATIONAL REGULATORY ELEMENTS
Inventor(s): Therese Ouellet, et al.
Application No.: 09/747,007
Docket Number: 411044.90030

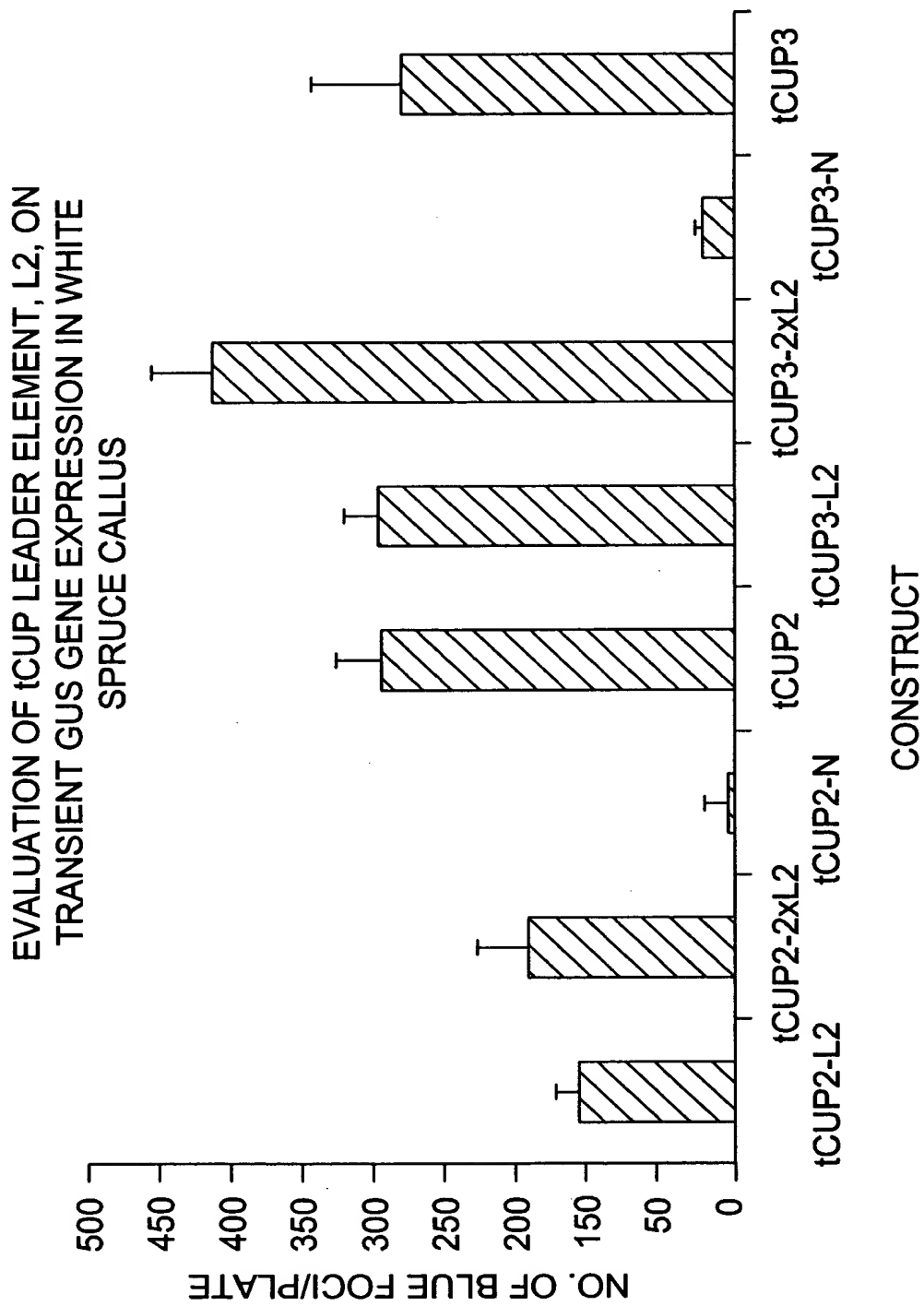


FIG. 9F

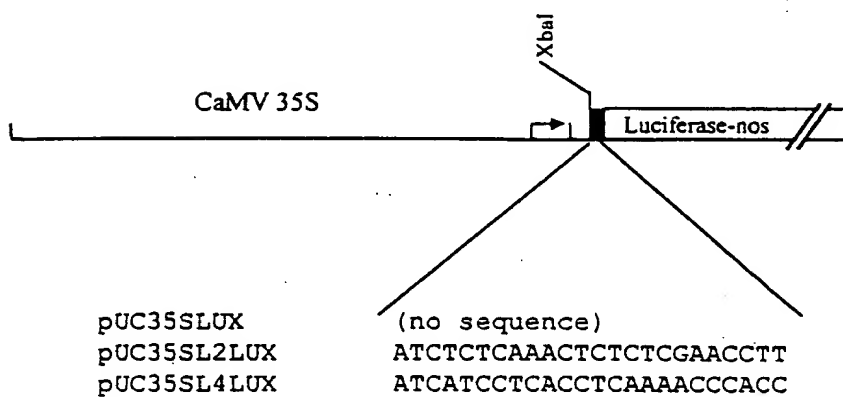


Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030



LEGEND:

- Vector sequence
- ▭ Luciferase reporter gene
- ▬ L2 or L4
- Start of transcription

FIGURE 10A



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

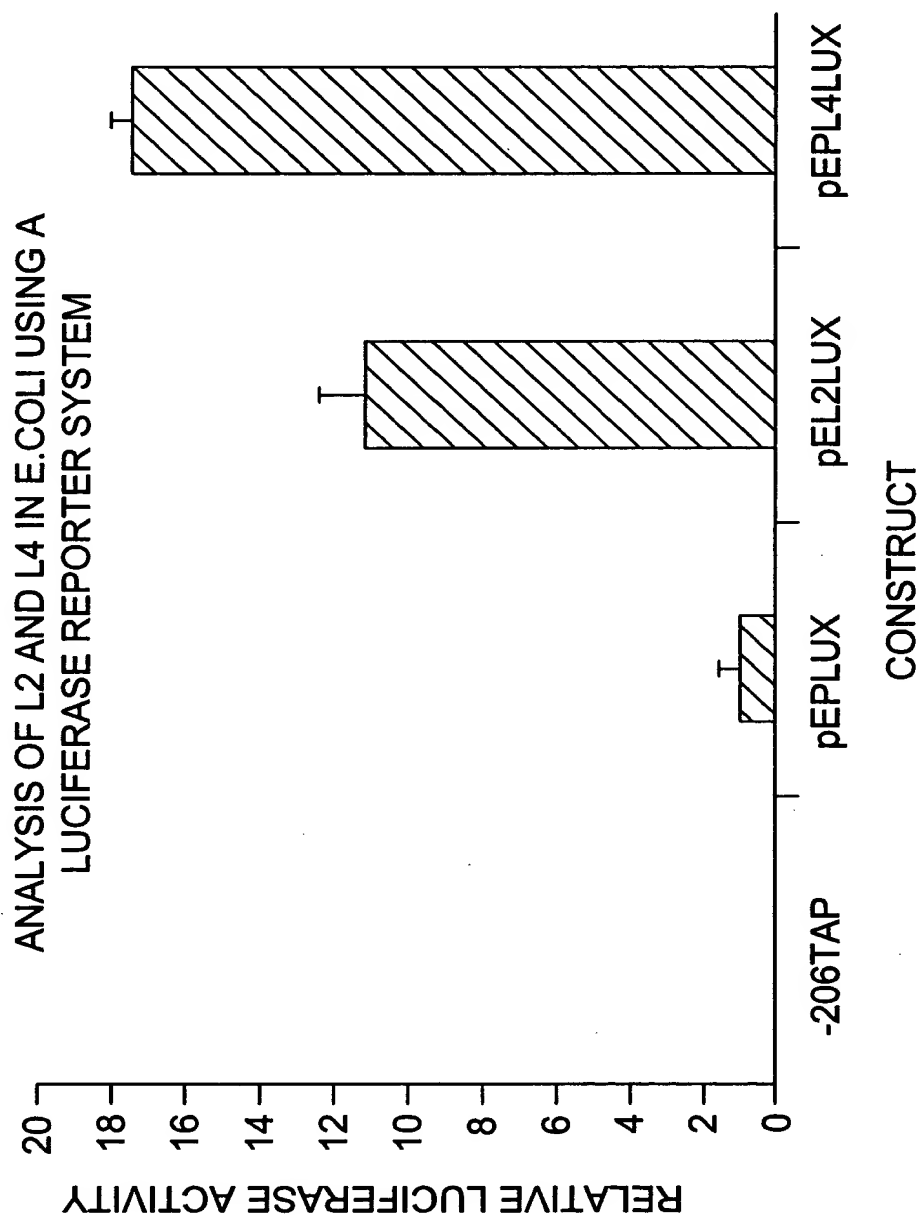


FIG. 10B



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

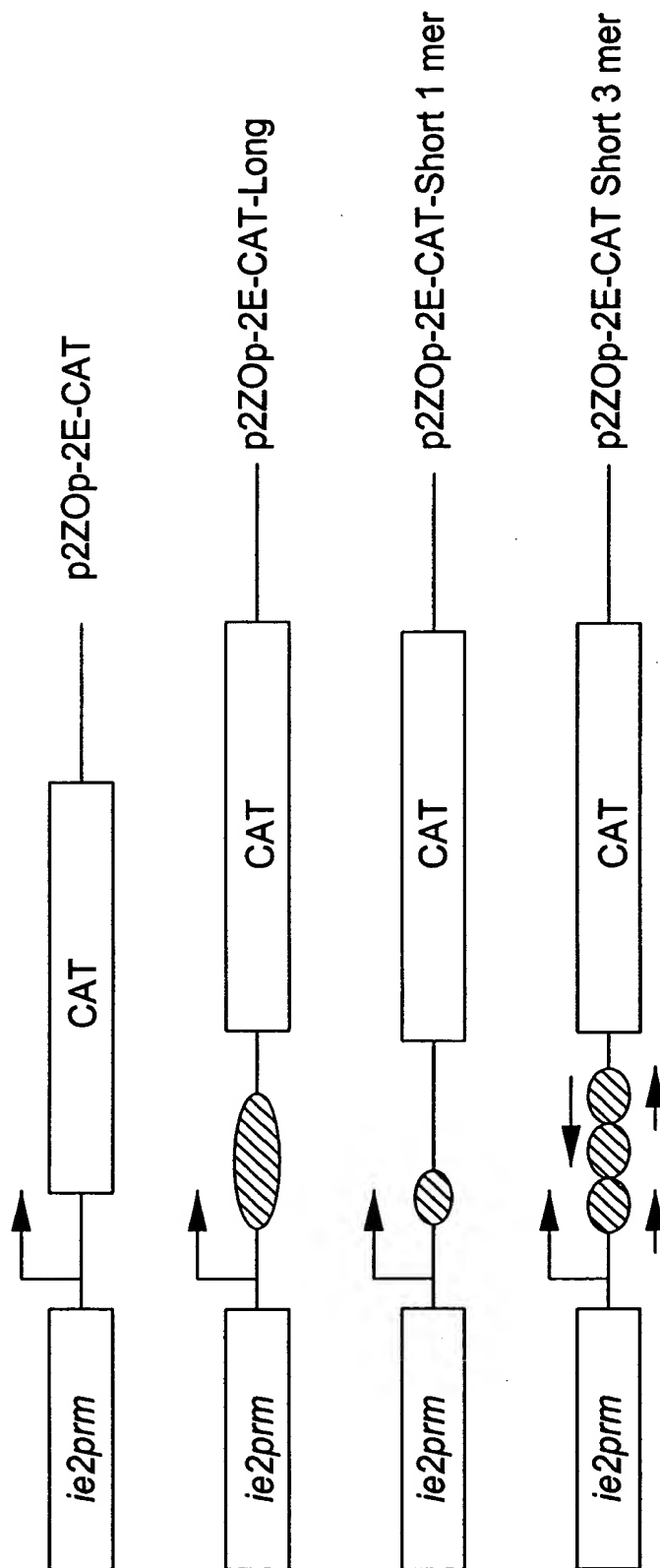


FIG. 11A



Title: TRANSLATIONAL REGULATORY ELEMENTS

Inventor(s): Therese Ouellet, et al.

Application No.: 09/747,007

Docket Number: 411044.90030

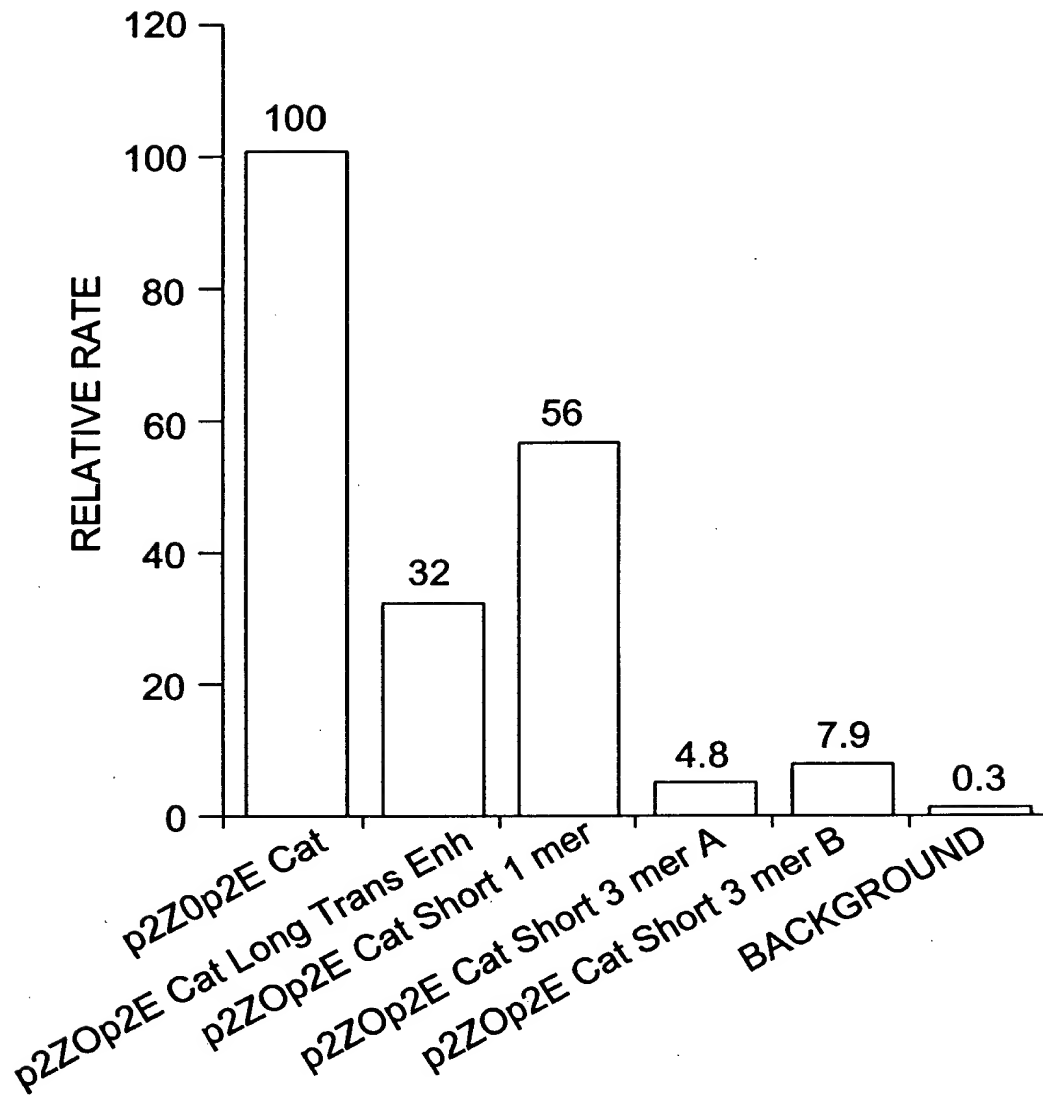


FIG.11B